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**Microcosms as Potential Screening
Tools for Evaluating Transport
and Effects of Toxic Substances**

W. F. Harris

ENVIRONMENTAL SCIENCES DIVISION
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Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Athens, Georgia 30605

MICROCOSMS AS POTENTIAL SCREENING TOOLS
FOR EVALUATING TRANSPORT AND EFFECTS
OF TOXIC SUBSTANCES

by

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FOREWORD

Environmental protection efforts are increasingly directed towards preventing adverse health and ecological effects associated with specific compounds of natural or human origin. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Environmental Systems Branch studies complexes of environmental processes that control the transport, transformation, degradation, and impact of pollutants or other materials in soil and water and assesses environmental factors that affect water quality.

Concern about environmental exposure to toxic substances has increased the need for accurate information on the transport, fate, and effects of trace contaminants in natural waters. One technique that shows promise as a useful and inexpensive tool for providing some of this information is the use of laboratory microcosms as ecosystems for the assessment of pollutant exposure to natural ecosystems. This report evaluates microcosms as research tools for providing accurate and reliable data concerning the potential transport, distribution, transformations, and persistence of inorganic and organic contaminants released to the environment.

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ABSTRACT

Terrestrial and aquatic microcosms were evaluated for use in research on environmental contaminants. Research completed in this project attempted to (1) evaluate relationships among size, complexity, stability, and replicability; (2) assess the similarity between microcosm results and actual environmental transport and effects; (3) identify and quantify system-level parameters that might be sensitive indicators of effects of chemical contaminants; (4) determine the relationship between system-level parameters measured in microcosms and in natural ecosystems; and (5) suggest protocols for establishing, maintaining, and interpreting results from microcosms. A number of experimental approaches using several microcosm designs and contaminants are reported. The use of terrestrial microcosm results was evaluated in conjunction with mathematical simulation models as a means of extending short-term experimental results to interpret conditions as they might occur in the natural ecosystem. Although microcosms offer an excellent experimental system, their application to toxic substance testing is not a straightforward matter. They are characterized by complex dynamics and counterintuitive responses just as is the ecosystem to which they are an analog. These factors notwithstanding, microcosms do offer an excellent means of studying specific aspects of contaminant behavior and ecosystem processes. With appropriate attention to the design of specific questions, answers to which are relevant to interpreting ecological transport and effects of contaminants, microcosms can be useful tools.

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TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT	vii
LIST OF TABLES	xiii
LIST OF FIGURES	xix
1.0 INTRODUCTION	1
2.0 SUMMARIES, CONCLUSIONS, AND RECOMMENDATIONS	5
2.1 AQUATIC STUDIES	5
2.1.1 Summary	5
2.1.2 Conclusions	6
2.1.3 Recommendations	8
2.2 TERRESTRIAL STUDIES	9
2.2.1 Summary	9
2.2.2 Conclusions	10
2.2.3 Recommendations	12
3.0 AQUATIC STUDIES	15
3.1 INTRODUCTION	15
3.1.1 State of the Art, Criteria, and Rationale	15
3.1.2 Summary of Research Strategy	17
3.2 SUMMARY OF RESULTS	24
3.2.1 Properties of Microcosms	24
3.2.1.1 Basic Ecological Characteristics of Pond Microcosms	24
3.2.1.2 Comparison of Microcosms with an Actual Pond	39
3.2.1.3 Replicability	40
3.2.1.4 Repeatability	49
3.2.1.5 Effects of Variations in Experimental Condition and Microcosm Design	53
3.2.2 Transport Studies	71
3.2.2.1 Arsenic	71
3.2.2.2 Chromium	95
3.2.2.3 Selenium	103
3.2.2.4 Mercury	115
3.2.2.5 Anthracene	129

	<u>Page</u>
3.2.3 Ecological Effects	142
3.2.3.1 Production/Respiration Ratios	143
3.2.3.2 Water Chemistry	148
3.3 DISCUSSION	155
3.3.1 Protocol for the Construction of Pond Microcosms	155
3.3.1.1 Collection of Components	157
3.3.1.2 Assembly of Microcosms	157
3.3.2 The Role of Pond Microcosms in Contaminant Research	160
3.3.2.1 Transport	164
3.3.2.2 Fate	168
4.0 TERRESTRIAL STUDIES	171
4.1 INTRODUCTION	171
4.1.1 State of the Art, Criteria, and Rationale	171
4.1.2 Summary of Research Strategy	172
4.2 SUMMARY OF RESULTS	175
4.2.1 Behavior of Heavy Metals in Forest Microcosms - Excised Tree Microcosms	176
4.2.1.1 Materials and Methods	176
4.2.1.2 Results and Discussion	180
4.2.2 The Behavior of Arsenic in Terrestrial Microcosms	196
4.2.2.1 Materials and Methods	198
4.2.2.2 Results and Discussion	199
4.2.2.3 Summary and Conclusions	204
4.2.3 Extraction of Nutrients from Intact Soils Cores	204
4.2.3.1 Materials and Methods	205
4.2.3.2 Results and Discussion	209
4.2.4 Hollow Fiber Membranes for Detecting Microbial and Nutrient Cycling Effects of Contaminants	214
4.2.4.1 Materials and Methods	214
4.2.4.2 Results and Discussion	217
4.2.4.3 Summary	219

	<u>Page</u>
4.2.5 The Effect of Substrate on Elemental Transport Through Terrestrial Microcosms	220
4.2.5.1 Results and Discussion	222
4.2.6 Functional Complexity and Microcosm Stability . .	226
4.2.6.1 Materials and Methods	229
4.2.6.2 Results	236
4.2.6.3 Discussion	239
4.3 SYNTHESIS OF TERRESTRIAL MICROCOSM RESULTS	239
4.3.1 Introduction	239
4.3.2 Minimal Monitoring Parameters	240
4.3.3 Size Effects	250
4.3.4 Substrate Effects	257
4.4 SIMULATION MODELING AND APPLICATION TO MICROCOSM AND FIELD DATA	261
4.4.1 Parameter Sensitivity Studies	264
4.4.1.1 Materials and Methods	264
4.4.1.2 Results	268
4.4.1.3 Discussion	291
4.4.2 Simulation of Chemical Transport in a Deciduous Forest Using Microcosm Calibration	295
4.4.2.1 Tree Microcosm Study and Model Parameterization	296
4.4.2.2 Field Study and Test Case Simulation . .	300
4.4.2.3 Discussion	304
4.5 DISCUSSION	306
4.5.1 The Role of Terrestrial Microcosms in Contaminant Research	306
4.5.1.1 Introduction	306
4.5.1.2 Measured Parameters	310
4.5.1.3 Replicability, Reproducibility	315
4.5.1.4 Accuracy	317
4.5.1.5 Problems	322
4.5.1.6 Recommendations	323

	<u>Page</u>
4.5.2 Protocol for Construction and Operation of Terrestrial Microcosms	324
4.5.2.1 Objectives	324
4.5.2.2 Rationale	324
4.5.2.3 Protocol 1	327
4.5.2.4 Protocol 2	329
5.0 LITERATURE CITED	335
APPENDIX A - Synopsis of Aquatic Experiments	351
APPENDIX B - Microcosm Biota	371
APPENDIX C - Project Publications	375

LIST OF TABLES

<u>Table</u>		<u>Page</u>
3.1	Comparison of water chemistry in microcosms, pond enclosures, and a pond	41
3.2	Coefficients of variation: Microcosm water chemistry . .	43
3.3	Coefficients of variation: Ecosystem metabolism	47
3.4	Coefficients of variation: Contaminant concentrations .	48
3.5	Arsenic concentrations in microcosm components - Comparison of Experiment 1 and Experiment 2	52
3.6	Mean nutrient concentrations: Experiment 1	56
3.7	Summary of water chemistry data: Experiment 1	57
3.8	Experiment 1 final arsenic concentrations (ppm)	61
3.9	Mean coefficients of variation (for 3 replicates) of chemical parameters in 7- and 70-liter microcosms	65
3.10	Coefficients of variation for arsenic concentrations (%) in Experiment 1	66
3.11	Temperatures of microcosm experiments	68
3.12	Gross primary production in fish microcosms	72
3.13	Water chemistry in fish microcosms after 12 weeks (all values are in ppm)	73
3.14	Summary of kinetic loss rate constants for microcosm water	77
3.15	Adsorption and desorption parameters for arsenic in microcosms (calculated from equation 1)	78
3.16	Arsenic concentrations and bioaccumulation ratios from the literature	82
3.17	Final mean arsenic concentrations (ppm): Experiment 2 .	89
3.18	Final arsenic mass balance - Experiment 2	93
3.19	Chromium kinetic loss rate constants, r , for microcosm water	99

<u>Table</u>	<u>Page</u>
3.20 Final Cr distribution	100
3.21 Final Se concentrations ($\mu\text{g/g}$ dry wt)	106
3.22 Final Se mass balance	109
3.23 Final Hg mass balance	119
3.24 Final Hg concentrations in microcosm components due to initial dose of 0.42 ng/ml (ppb) in water	120
3.25 Final ^{14}C activity in microcosm components twelve weeks after addition of 10.4×10^6 cpm as anthracene . .	134
3.26 ^{14}C budget	136
3.27 Percentage distribution of radioactivity in extracts . .	137
3.28 Effects of arsenic on microcosm water chemistry: Experiment 2	152
3.29 Inorganic nitrogen concentrations following arsenic treatment: Experiment 2	154
3.30 Summary of bioaccumulation ratios	163
4.1 Chemical and physical characteristics of experimental soils	174
4.2 Source of heavy metals (Pb, Zn, Cd, Cu) deposited as contaminated litter and as baghouse dust on forest microcosms	178
4.3 Heavy metal concentrations in litter, soil, and vegetation (<i>Acer rubrum</i>) components of forest microcosms (N=3) . . .	181
4.4 Total leachate export of heavy metals from forest microcosms over the 20-month experiment	183
4.5 Heavy metal enrichment ratios for forest microcosms . . .	185
4.6 Initial litter and soil nutrient pools in contaminated and control microcosms	186
4.7 Total dissolved nutrient export from contaminated and control forest microcosms over the 20-month experiment .	188
4.8 Final litter nutrient pools and total changes in nutrient pools over the 20-month experiment	189

<u>Table</u>		<u>Page</u>
4.9	Mean nutrient concentrations of forest microcosm components at experiment termination	191
4.10	Comparison of average CO ₂ efflux rates (mg CO ₂ /microcosm/day) of contaminated and control microcosms during spring and summer of the two years of observations	194
4.11	Simple linear regression of cumulative CO ₂ efflux (mg) upon time (days) for contaminated and control microcosms for the two years of experimentation	195
4.12	Microbial parameters in soil of contaminated and control microcosms at the end of the 20-month experiment	197
4.13	Final As concentrations of As-amended forest and grassland soil cores ($\pm$ S.E., n=3)	200
4.14	Aqueous efflux of Ca and NO ₃ -N from forest and grassland soil cores amended with As ($\pm$ S.E., n=18)	202
4.15	Extraction of nutrients from intact soil cores using various extractant solutions [μ g/g ($\bar{X} \pm$ S.E.)].	207
4.16	Mean nutrient concentrations (μ g/ml) of soil solutions extracted from 10- and 20-cm depths with hollow fibers and lysimeters ($\pm$ 1 S.E., n=10)	218
4.17	Cumulative elemental transport (mg) through terrestrial microcosms of three substrates over a 118-day experiment	223
4.18	Rates of essential element transport (mg/microcosm/week) through terrestrial microcosms constructed of three substrates (N = 18 weekly observations)	225
4.19	Probability (Z) that successive observations of elemental transport through any microcosm unit differ by > 100% (N = 17/replicates)	227
4.20	Rankings of functional complexity and ecosystem stability based on monitoring of CO ₂ and total Ca export in soil leachate	234
4.21	Summary of microcosm experiments and corresponding parameters	241
4.22	Comparison of variability among elements leached from terrestrial microcosms	242
4.23	Comparison of variability among elements leached from terrestrial microcosms	244

<u>Table</u>	<u>Page</u>
4.24 Alpha values for the significance of treatment (toxic substance) effect in eight experiments	246
4.25 Average "t" values compared among elements	247
4.26 Correlation coefficients for Ca and Mg export	249
4.27 Microcosm size comparison on intact cores in Grassland I where size was a variable	251
4.28 Microcosm size comparison on intact cores in Grassland II	252
4.29 Comparison of variability of Ca and NO ₃ between small and large microcosms	254
4.30 Comparison of CO ₂ efflux of small (10x10 cm) and large (30x15 cm) intact core microcosms	255
4.31 Calculated F values for treatment effect in two microcosm experiments	256
4.32 Homogenized grassland soil cores and intact grassland cores were compared in Grassland II	259
4.33 Comparison of variability of Ca and NO ₃ between intact and homogenized microcosms	260
4.34 Some attributes of coupled models describing carbon, water, and chemical dynamics in the soil plant-litter system	263
4.35 Standard parameter values and tested values for sensitivity analyses	265
4.36 Model output variables considered in this study	267
4.37 Subjective evaluation of parameter effects on total chemical level and distribution in soil, litter, and plant components	293
4.38 Comparison of water, carbon, and chemical data for the microcosm and modeling studies	298
4.39 A comparison of initial simulation, experimental data, and simulation results from this study for Crooked Creek Watershed	302
4.40 Uses that various microcosm units have for specific screening applications	308

<u>Table</u>		<u>Page</u>
4.41	Microcosm test systems, substances tested, and citations	311
4.42	Population and system level parameters measured in soil microcosms, forest microcosms, and forest under control and chemical stress conditions	314
4.43	Indices of microbial activity measured at termination of a microcosm experiment using complex heavy metal emissions from a lead smelter	316
4.44	Comparison of three parameters measured in control replicates of three soil core microcosm experiments . . .	318
4.45	Comparison of extrapolated annual export among substrates	321

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
3.1	Production and respiration rates in microcosms from Experiment 9	28
3.2	Production/respiration ratios from three microcosm experiments	30
3.3	Dissolved organic carbon in microcosm water from Experiment 1 (mean of three 70-liter sediment microcosms), Experiment 2 (mean of three control microcosms), and Experiment 9 (mean of two microcosms) .	31
3.4	Dissolved oxygen and pH in microcosm water, Experiment 2	33
3.5	Nitrate, ammonium, and total phosphorus concentrations in microcosm water, Experiment 9	34
3.6	Nitrate, ammonium, and total phosphorus concentrations in microcosm interstitial water, Experiment 9.	36
3.7	Coefficients of variation of conductivity, dissolved oxygen, and pH, Experiment 2	44
3.8	Coefficients of variation of nitrate, ammonium, phosphate, total phosphorus, and dissolved organic carbon, Experiment 2	45
3.9	Dissolved arsenic concentrations, Experiments 1 and 2	51
3.10	Dissolved oxygen concentrations, Experiment 1	54
3.11	pH, Experiment 1	55
3.12	Final algal biomass, Experiment 1	58
3.13	Final chlorophyll-a concentrations, Experiment 1	59
3.14	Arsenic concentrations in water, Experiment 1	60
3.15	Equilibration of dissolved oxygen in 7- and 70-liter sediment microcosms, Experiment 1	62
3.16	Equilibration of pH in 7- and 70-liter sediment microcosms, Experiment 1	63

<u>Figure</u>		<u>Page</u>
3.17	Final distributions of arsenic among microcosm components, Experiment 1	79
3.18	Regression of snail arsenic concentrations ($\mu\text{g As/g dry wt}$) on substrate arsenic concentrations, Experiment 1	81
3.19	Dissolved arsenic concentrations, Experiment 2	87
3.20	Dissolved arsenic concentrations, Experiments 2 and 3	88
3.21	Sediment arsenic profiles, Experiment 2	91
3.22	Dissolved chromium concentrations, Experiment 4	97
3.23	Sediment chromium profiles, Experiment 4	101
3.24	Dissolved ^{75}Se concentrations, Experiment 6	107
3.25	Sediment ^{75}Se concentrations, Experiment 6	108
3.26	^{75}Se concentrations in algae and <u>Elodea</u> tips, Experiment 6	110
3.27	^{75}Se concentrations in <u>Elodea</u> stems, Experiment 6	111
3.28	^{75}Se concentrations in <u>Elodea</u> roots, Experiment 6	112
3.29	^{75}Se concentrations in fish and invertebrates, Experiment 6	114
3.30	Dissolved ^{203}Hg concentrations, Experiment 7	117
3.31	Sediment ^{203}Hg concentrations, Experiment 7, top cm only	118
3.32	<u>Elodea</u> ^{203}Hg concentrations, Experiment 7, replicate C1	122
3.33	<u>Elodea</u> ^{203}Hg concentrations, Experiment 7, replicate C2	123
3.34	Bioaccumulation ratios for ^{203}Hg in <u>Elodea</u> tips and stems, Experiment 7	124
3.35	Snail tissue ^{203}Hg concentrations, Experiment 7	125
3.36	Bioaccumulation ratios for ^{203}Hg in snail tissue, Experiment 7	126

<u>Figure</u>		<u>Page</u>
3.37	Zooplankton ^{203}Hg concentrations, Experiment 7, replicate C1	128
3.38	^{14}C activity in microcosm water, Experiment 8	133
3.39	Distribution of ^{14}C activity in extracts from microcosm water, Experiment 8	138
3.40	Comparison of ^{14}C distribution in extracts from microcosm water and distilled water, Experiment 8	140
3.41	Net production and nighttime community respiration in microcosms at three arsenic levels, Experiment 2	144
3.42	Production/respiration ratios in microcosms at three arsenic levels, Experiment 2	146
3.43	pH, Experiment 2	149
3.44	Dissolved oxygen concentrations, Experiment 2	150
3.45	Alkalinity, Experiment 2	151
3.46	Dissolved organic carbon, Experiment 2	153
3.47	Total phosphorus concentrations, Experiment 2	156
4.1.	Effect of heavy-metal contamination on extractable soil nutrients by the intact and slurry methods.	192
4.2.	Mean weekly Ca efflux from untreated forest and grassland soil cores	203
4.3.	Nutrient pools extracted from Emory and Captina soils by leaching intact and homogenized soil cores, and by the slurry method	211
4.4.	Schematic diagram of an intact soil core containing devices for extracting in-situ soil solution	216
4.5.	Schematic of glass manifolds for field use of polycarbonate hollow fibers.	221
4.6.	Cross-section of old-field microcosm showing two system-level monitoring points of CO_2 efflux and nutrient leachate	230
4.7.	Example of time series of CO_2 efflux from pasture microcosms	231

<u>Figure</u>		<u>Page</u>
4.8.	Mean DOC (-----) and NO ₃ -N (——) concentration in leachate (± 1 S.E., n = 13)	233
4.9.	Relative lead distribution in plant, litter, and soil components with change in lead distribution coefficient (K_d)	270
4.10.	Influence of zinc K_d on zinc transport in the vegetation: (a) leaf-to-stem phloem, and (b) stem-to-leaf xylem	271
4.11.	Relative zinc distribution in plant, litter, and soil components with change in zinc solubility (SP)	272
4.12.	Relative lead distribution in plant, litter, and soil components with change in diffusion coefficient of lead (DL)	274
4.13.	Relative zinc distribution in plant, litter, and soil components with change in litter decomposition rate constant (DMAX)	275
4.14.	Relative chemical distribution in plant, litter, and soil components with change in leaf-to-stem phloem resistance (LSPHLO) for (a) lead, and (b) zinc	277
4.15.	Relative chemical distribution in plant, litter, and soil components with change in standard root respiration rate (RRESTD) for (a) lead, and (b) zinc	279
4.16.	Influence of standard stem respiration rate (SRESTD) on sugar translocation in stem-to-root phloem	281
4.17.	Relative chemical distribution in plant, litter, and soil components with change in leaf area weight ratio (ARI) for (a) lead, and (b) zinc	282
4.18.	Influence of root radius (R) on root uptake of lead during the sunlight period	284
4.19.	Relative chemical distribution in plant, litter, and soil components with change in maximum leaf storage (L MAX) for (a) lead, and (b) zinc	286
4.20.	Relative chemical distribution in plant, litter, and soil components with change in leaf permeability to chemicals (PERM) for (a) lead, and (b) zinc	287

<u>Figure</u>		<u>Page</u>
4.21.	Relative chemical distribution in plant, litter, and soil components with change in leaf cuticle thickness (FILM) for (a) lead, and (b) zinc	289
4.22.	Relative zinc distribution in plant, litter, and soil components with change in root chemical conductivity (CONDOC)	290
4.23.	Relative zinc distribution in plant, litter, and soil components with change in atmospheric CO ₂ (CO ₂ X)	292
4.24.	Replicability of two parameters monitored in microcosms. (A) - CO ₂ efflux; (B) - DOC efflux	319
4.25.	Protocol 1 soil core excised intact from a forest ecosystem	328
4.26.	Protocol 2 grassland microcosm excised from a <u>Festuca</u> -dominated pasture	331
4.27.	Protocol 2 forest microcosm containing an <u>Acer rubrum</u> sapling excised from a mesic hardwood forest	332

1.0 INTRODUCTION

There is continuing concern about the environmental transport, fate, and effects of toxic substances in the biosphere. Legislation now requires, in part, a determination of the environmental fate of chemicals before large-scale production, marketing, and dissipation can occur. Implementation of this legislation requires reliable, adaptable, and inexpensive research tools capable of evaluating environmental transport, fate, and effects of trace contaminants. Sensitivity and precision are requirements of the research techniques used to determine the effects and distribution of chemicals released to the environment. A recent survey of testing procedures that have proven useful in the identification and quantification of the environmental transport of chemicals revealed that laboratory microcosm experiments can provide substantial information on the transport and accumulation of chemicals in the environment (Witherspoon et al. 1976). We define microcosm as any confined ecosystem or part thereof that may be subjected to laboratory controls due to its reduction in size or complexity. Such systems may be created artificially or they may represent portions of natural ecosystems which can be brought into the laboratory for study. The report also indicated that there currently exist no standardized microcosms for the determination of chemical fate, and that the reliability and validity of results obtained from microcosm studies have not been fully determined.

Testing procedures (possibly including microcosms) need to be designed expressly for the determination of chemical fate in order to

give results which are relevant to actual environmental occurrences. In this approach to microcosm research we initially recognized that the development of a single, complex microcosm for the assessment of chemical fate in numerous ecosystems is not realistic. Complex microcosms, which contain all the major functional groups of natural ecosystems, can be useful in identifying chemical accumulation, but are insensitive for measuring rates of synergistic and/or competitive transport processes. Microcosms of reduced complexity containing the essential functional components of natural ecosystems can be used to estimate the chemical fate in isolated ecosystem components, and are suitable for measuring a defined number of rate processes over short time periods. These synthetic microcosm systems do provide for estimation of rate constants for identifiable transport processes in specific environments.

Several problems exist in the use of microcosm results for both the description and prediction of environmental behavior of pollutants. First is the problem of applying results from processes measured in isolation to the more complex natural ecosystems. Secondly, the relationships among microcosms of different size and structure have not been evaluated. Finally, there are no estimates of confidence limits on parameter measurements and of microcosm reproducibility.

The work undertaken in this project was designed to provide some insight to the above questions through the following objectives:

- (1) To evaluate the relationships among microcosm size, complexity, and replicability to application of microcosm results in both

characterizing and mathematically predicting environmental transport, accumulation, and persistence of contaminants.

(2) To determine the realism and validity of microcosm results in relation to the ability of these results to reflect actual environmental occurrences.

(3) To utilize results obtained in objectives 1 and 2 in evaluating microcosms as potential "screening tools" for trace toxic contaminants released from industrial sources.

The products of this research initially were conceived to include (1) a greater understanding of the ecological processes governing microcosm behavior; (2) an evaluation of microcosms as research tools for providing accurate and reliable data concerning the potential transport, distribution, transformations, and persistence of both inorganic and organic contaminants released to the environment; and (3) an evaluation of microcosms as "screening tools" for use by industry and federal agencies in assessing potential environmental transport and distribution of trace contaminants.

2.0 SUMMARIES, CONCLUSIONS, AND RECOMMENDATIONS

2.1 AQUATIC STUDIES

2.1.1 Summary

A protocol has been developed for the construction and operation of pond microcosms for use in research on environmental contaminants. The microcosm components (water, sediment, and a complete biotic community) are taken directly from a natural pond, assembled in 70-liter glass aquaria, and held in an environmental chamber at a constant temperature on a 12-hr-light:12-hr-dark cycle. Pond microcosms equilibrate (achieve internal constancy) within 6 to 8 weeks, and remain stable for several months or even longer. The ecosystems which develop in the microcosms are ecologically similar to the parent ecosystem. Variations between replicate microcosms are small (coefficients of variation generally less than 20%) for most measured parameters. The protocol has been tested and refined through a series of experiments on the environmental transport and fate of arsenic, chromium, selenium, mercury, and anthracene (a polycyclic aromatic hydrocarbon). Pond microcosms are too complex for analysis and measurement of individual transport processes and degradation pathways, but they do provide information on overall net rates and pathways under realistic environmental conditions. These microcosms could also be used to study effects of contaminants on community interactions and ecosystem processes.

2.1.2 Conclusions

- (1) Pond microcosms constructed according to the protocol described herein are realistic simulations of shallow pond ecosystems.
- (2) Pond microcosms achieve a state of internal constancy within 6 to 8 weeks, and persist in this condition for several months or longer.
- (3) Pond microcosms are about as replicable as most other biological entities. Coefficients of variation among replicate microcosms are generally below 20% for most measured parameters.
- (4) Experiments on contaminant transport in microcosms are repeatable. Results are not highly dependent on the time of year the microcosms are constructed.
- (5) Smaller (7-liter) microcosms behave similarly to the 70-liter microcosms described in the protocol, but are less stable, more easily disturbed, and more difficult to sample than the larger microcosms.
- (6) Substrate type greatly influences contaminant transport in pond microcosms.
- (7) Small fish are compatible with a stable and persistent microcosm community, although they may affect community composition and metabolism. Snails and crayfish, on the other hand, have a significant impact on microcosm ecology.
- (8) Results of two experiments on arsenic transport were consistent with each other and with published field data.

- (9) The loss of a contaminant from microcosm water can be described by a first-order kinetic constant "r." Of the five contaminants tested, values of r were highest for mercury (implying rapid sorption by sediments) and lowest for selenium (implying greater potential for hydrologic transport of this metal).
- (10) Most of the contaminants tested were concentrated in organisms to about 10^2 to 10^3 times the equilibrium water concentration. Mercury was an exception, with bioaccumulation ratios (concentration in organism/concentration in water) of 10^4 to 10^5 ; this is consistent with the known bioaccumulation tendencies of this metal. Bioaccumulation ratios are most useful for comparisons between contaminants; extreme values appear to be reliable indicators of highly bioaccumulative substances.
- (11) Microcosm experiments can provide information on the net degradation rate of a compound in a whole ecosystem and the identity, quantity, and persistence of derivatives. Major degradation processes may sometimes be inferred from the data, but confirmation must come from experiments with simpler systems in which the hypothesized pathways are isolated from competing processes.
- (12) Although the primary goal of the project was to develop microcosms for testing contaminant transport and fate, pond microcosms can also be used to test for effects on whole ecosystems. Possible "pulse points" for indication of ecosystem dysfunction include the concentrations of nutrients in interstitial water and the ratio of primary production to total ecosystem respiration.

2.1.3 Recommendations

- (1) The major advantage of pond microcosms over other contaminant testing tools is their realistic simulation of an entire aquatic ecosystem. Therefore, microcosms are most useful in the advanced stages of contaminant testing, when accurate predictions of the overall fate and effects of a substance are required, rather than in the earlier screening stages.
- (2) For accurate simulation of a particular aquatic ecosystem, microcosm components (water, sediment, and biota) should be collected from that ecosystem.
- (3) Whenever possible, contaminants to be tested should be labeled with an appropriate radioisotope. This reduces sample sizes necessary for analysis, improves detection limits, and permits identification of derivatives of the parent compound.
- (4) Microcosm experiments integrate, and thereby obscure, individual transport processes and degradation pathways. Single-species experiments facilitate the analysis of individual processes, but provide no direct means of assessing overall contaminant behavior. These two complementary experimental approaches are most fruitful when used in combination.
- (5) Microcosm results can be used to identify weaknesses in analytical models and can provide an overall picture of contaminant behavior when models fail. Models, on the other hand, can be used in conjunction with data from simplified experimental systems to assist in the interpretation of microcosm results.

- (6) Further comparisons are needed between microcosms and the natural ecosystems they represent. Comparisons of contaminant transport and fate in microcosms and natural ecosystems are also needed.
- (7) The feasibility of improving the accuracy of simulation through the use of larger systems, natural light and temperature regimes, and continuous nutrient input should be investigated.

2.2 TERRESTRIAL STUDIES

2.2.1 Summary

Terrestrial microcosms were evaluated for use in research on environmental contaminants. Research completed in this project attempted to: (1) evaluate relationships among size, complexity, stability, and replicability; (2) assess the similarity between microcosm results and actual environmental transport and effects; (3) identify and quantify system-level parameters which might be sensitive indicators of effects of chemical contaminants; (4) determine the relationship between system-level parameters measured in microcosms and in natural ecosystems; and (5) suggest protocols for establishing, maintaining, and interpreting results from terrestrial microcosms. In addition to a number of experimental approaches using several microcosm designs and contaminants (principally trace metals), the use of microcosm results was evaluated in conjunction with mathematical simulation models as a means of extending short-term experimental results to interpret conditions as they might occur in the natural ecosystem. While microcosms offer an excellent experimental system, their application to toxic substance testing is not a straightforward matter. They are characterized

by complex dynamics and counter-intuitive responses just as is the ecosystem to which they are an analog. These factors notwithstanding, terrestrial microcosms offer an excellent means of studying specific aspects of contaminant behavior and ecosystem processes. With appropriate attention to the design of specific questions, answers to which are relevant to interpreting ecological transport and effects of contaminants, terrestrial microcosms can be useful tools. As a panacea to toxic substance testing, they leave many questions unanswered.

2.2.2. Conclusions

(1) The suite of seven chemical species (Mg, Ca, DOC, K, $\text{NO}_3\text{-N}$, P, $\text{NH}_4\text{-N}$) is too large a number to be practicable in a routine screening program. On the basis of variability and sensitivity tests, it would appear that $\text{NO}_3\text{-N}$ plus either Ca or Mg would serve as a practical pair of chemical species for a screening program.

(2) Based on nutrient variability and sensitivity, the effect of size (10 x 10 cm vs 30 x 25 cm) was significant ($P < 0.01$). With few exceptions, the large (30- x 25-cm) microcosms showed greater concentrations of leachates.

(3) With respect to effects of size on variability, no unambiguous pattern was apparent; indeed, in many cases, the larger microcosms showed greater variability both through time and between replicates.

(4) Size effects also appeared to play a role in CO_2 measurements. Large microcosms showed smaller coefficients of variation with great consistency.

(5) Large microcosms (30 x 25 cm) showed a clearer treatment effect (larger F ratio) than did small microcosms. Smaller size microcosms, however, have sufficient sensitivity to detect an effect. Choice of size will require greater operating experience to decide the relative advantages of using a few large microcosms versus a larger number of smaller microcosms.

(6) Because the major argument for using a homogenized substrate is the potential for decreased variability, and because our data indicate that there is not a consistent trend for variability with this substrate, there seems little argument for use of homogenized substrate.

(7) Forest soils used in these experiments were more sensitive (particularly Ca loss) than grassland soils.

(8) Others have identified the need to relate quantitatively the data from microcosms to chemical behavior in natural ecosystems. Our test case application in this study showed that such a simulation is limited by the field relevance of the data set. Microcosm data provide one means of model parameter estimation; however, the parameter values may not be representative of the natural ecosystem.

(9) Forest microcosms treated with heavy metals in smelter bag-house dust yielded final results qualitatively consistent with observed conditions in the immediate environs of the smelter.

(10) Techniques were developed for measuring soil nutrients which appear to be more sensitive to detecting subtle effects on soil chemistry than conventional slurry techniques.

(11) Long-term monitoring of nutrient export dynamics in grass microcosms (soil intact) showed that seasonal changes in sensitive parameters might obscure contaminant effects on nutrient dynamics. Therefore, a control series should be included in each experimental series.

(12) Based on detailed analysis of microcosm metabolism (CO_2 efflux) and responses to a chemical perturbation (Cd applied to intact grassland microcosms), it was experimentally shown that increasing stability appeared to accompany increased complexity. This finding is important not only to further use of microcosms in experimental ecology but also to use of microcosms in toxicant screening since stability and complexity are fundamental attributes of ecosystems. Therefore, how these attributes compare between microcosm and natural systems as well as how these attributes influence toxicant behavior is important.

2.2.3 Recommendations

(1) Microcosms are isolated portions of natural ecological assemblages. Research to date shows that on at least a qualitative basis there is a correspondence between transport and effects of toxicants in microcosm versus natural ecosystems. Furthermore, terrestrial microcosms from different temperate ecosystems (mesic forest vs old-field grassland) are qualitatively, if not quantitatively, similar in their behavior with respect to baseline conditions and in response to toxicant input. There should be no reason to expect a single terrestrial microcosm design to show exact correspondence with nature. The variety of nature and particular properties of microcosms both point to this

inescapable conclusion. Therefore, the utility of microcosms in evaluating potential toxicants lies in how will we ask questions about ecological effects and transport. As with any rigorous scientific study, the value of the information derived is dependent upon how carefully the initial hypothesis is structured. Therefore, there remains the need to decide upon that set of important questions about the ecological transport and effects of a potential toxicant. The experimental design will then follow naturally. Experimental ecologists, indeed biologists generally, have used microcosm-like laboratory systems long before the recognition of their applicability to toxicant screening.

(2) The exercise undertaken in this project to employ mathematical models based on physicochemical and biological data in microcosm studies as a means to develop models for environmental behavior of chemicals suggests additional areas for analysis. There is a need to determine how best to use microcosm data to evaluate model algorithms, to validate models, to estimate model parameters in a manner representative of the real world, and to combine to simplified representations (microcosms and simulation models) in order to gain insights into longer time-frame behavior.

(3) One feature consistently exhibited by terrestrial microcosms subjected to contaminants was the accelerated loss of nutrients (Ca, $\text{NO}_3\text{-N}$, and others on a less consistent basis). Nutrient loss generally accompanies disturbance, especially physical disturbances, of terrestrial ecosystems. The exact mechanisms underlying the greater loss of nutrients are not completely known. Furthermore, because of the cyclic nature of nutrient metabolism in terrestrial ecosystems,

there is no reason to expect a single mechanism or set of mechanisms to be responsible. Therefore, a major uncertainty to the use of terrestrial microcosms involves studies of nutrient metabolism processes to determine exactly the causal factors underlying the accelerated nutrient loss. This is essential for two reasons: (1) while the phenomenon is general, it is logical to expect it to be accepted as an index of ecological effects without being in a position to explain the origin of the phenomenon; and (2) if the mechanisms leading to excess nutrient leakage can be identified and the number of physicochemical factors involved are not extensive, this knowledge may suggest very simple experimental systems applicable to screening purposes.

(4) Finally, while it is possible to design a biological system in the laboratory which has many properties in common with the real world and which demonstrates an effect when perturbed with a foreign substance, there remain questions about recovery, chronic vs acute exposure, and ecological significance of any effect demonstrated in the laboratory. Therefore, there is a need to calibrate real-world responses with microcosm responses. Such a calibration assumes that an acceptable level of ecological behavior can be agreed upon. This is not a trivial assumption.

3.0 AQUATIC STUDIES

3.1 INTRODUCTION (J. M. Giddings)

3.1.1 State of the Art, Criteria, and Rationale

Shortly before this project was initiated, a review of the state of the art of assessing the environmental fate and transport of chemicals was completed (Witherspoon et al. 1976). The review indicated that simplified microcosms, such as that developed by Metcalf and his associates (Metcalf et al. 1973), were suitable for measurement of rate processes over short time periods. Three critical problems were identified:

- (1) REALISM: the difficulty of applying results obtained in simplified systems to complex natural environments;
- (2) REPRODUCIBILITY: the lack of information on the comparability of data from similar but nonidentical microcosms; and
- (3) REPLICABILITY: the inability to set confidence limits on parameters measured in microcosms.

The present study was undertaken to resolve these three problems.

The type of microcosm which formed the basis for discussion in the state-of-the-art review, and which was described in our first proposal, was the model food chain, of which the Metcalf microcosm is the best-known example. Measurements of the bioaccumulation and biodegradation of chemicals in model food chains can be used to rank a group of related chemicals according to their food chain mobility, potential for biomagnification, and degradability. Good replication (i.e., narrow confidence limits on measured parameters) is necessary for consistent

rankings. In principle, Metcalf microcosms are reproducible and standardizable. Isensee (1976) has measured the replicability of Metcalf-type microcosms and found them to be comparable to other types of biological experiments. If Isensee's results are typical, then Metcalf microcosms satisfy most of the criteria for a screening test: replicability, rapidity, and simplicity. They are not representative of real ecosystems, but an accurate simulation of nature is not essential in a screening test. The objective of screening is to identify the most hazardous substances out of a large group; this is quite different from understanding the behavior of a chemical in an actual environment.

When a compound is determined (through the screening program) to be hazardous, it is necessary to arrive at a more detailed prediction of its fate and effects under natural conditions. To make predictions concerning a given environment, it is important to simulate that environment as closely as possible. Model food chain microcosms differ from real ecosystems both structurally and functionally. They lack important ecosystem components, especially detritus and the detrital food web. They cannot be made complex enough to include the diversity of transport pathways and opportunities for degradation which would be found in a natural ecosystem. Recycling of essential elements does not occur, nor do many other interactions responsible for homeostasis in a natural ecosystem. Most model food chains are unstable and short-lived, unlike ecosystems, which are self-regulating and persistent through time. Because of these differences, model food chains are incapable of accurately simulating chemical transport and fate in real ecosystems.

The three problems identified by Witherspoon et al. (1976), listed above, do not all apply to one testing system. Reproducibility and replicability are critical in a screening test, while realism is of greater concern when detailed predictions are required. The research reported here centered on the problem of realistic simulation of a selected aquatic environment, although reproducibility and replicability were also investigated. We did not re-invent the Metcalf microcosm, but concentrated instead on maximizing the predictive capabilities of a more complex, more naturalistic, microcosm design.

3.1.2 Summary of Research Strategy

The immediate goal at the outset of the project was to design a microcosm which would provide a relatively constant biotic and abiotic environment in which to examine the behavior of contaminants. Stability, in the sense of internal constancy, was a prime objective. It was also deemed essential that the microcosm produce realistic data, i.e., data consistent with observations in the field. Beyond this, replicability and reproducibility of results and convenience of operation were desired.

Our first task was to select appropriate microcosm components. Aquatic organisms were collected from local ponds and streams and tested for their ability to survive under laboratory conditions. We observed that natural communities of filamentous algae and their associated biota were often little affected by transfer from their native habitats to static aquaria. Spring water was found to be more acceptable to some organisms than standard reference water. Several potential substrate types were compared, and two (lake sediment and silica sand) were selected for further study.

We assembled prototype 7-liter (1.85-gal) microcosms containing spring water and filamentous algal communities, with and without lake sediment. A third microcosm, with an algal culture (Scenedesmus abundans) instead of the natural community, was also constructed. The three systems were placed in a growth chamber and observed for six weeks. The system containing spring water, sediment, and a natural community was found to be the most stable, and this design was selected for the first major experiment.

Experiment 1 (February-April 1976) was intended to examine the ecological properties of the microcosms and to establish basic procedures for set-up, monitoring, and sampling. Two aquarium sizes [7- and 70-liter (1.85- and 18.5-gal)] and two substrate types (lake sediment and sand) were compared. After a seven-week equilibration period, a radiolabeled test compound (sodium arsenate as $\text{Na}_2\text{H}^{74}\text{AsO}_4$) was added to the water in subtoxic concentrations, and its distribution was followed for five weeks. The microcosms were found to be stable, replicable, and persistent, and gave results on arsenic transport which were consistent with published field data. Size had no significant effect on the mean values of most parameters measured, but 70-liter microcosms were more replicable and equilibrated sooner than 7-liter microcosms. Substrate type, as expected, had a marked influence on water chemistry, biological productivity, and arsenic transport. Seventy-liter microcosms with lake sediment were the most satisfactory in terms of stability, replicability, and longevity, and this design was adopted for further work. The results of this experiment were summarized in an open-literature publication (Giddings and Eddlemon 1977).

In July 1976, we constructed 12 70-liter microcosms for Experiment 2. These were similar to the 70-liter sediment microcosms in the first experiment, except that the sediment was not dried and homogenized before use, and a macrophyte community was used instead of an algal community. During the first two months of the experiment we refined and extended our operating techniques, and gathered data by which to evaluate replicability of the microcosms. After an equilibrium had been reached, nine of the microcosms were treated with radiolabeled sodium arsenate at 0.066 ppm (approximately the same as the first experiment), at 11.5 ppm, and at 143 ppm. Arsenic distribution was studied as in the first experiment, and effects of arsenic on production, respiration, nutrient concentrations, and community structure were observed.

Concurrently with the microcosm experiment, a field experiment (Experiment 3) on the transport and effects of arsenic was conducted in a shallow pond. Six 60- x 120-cm Plexiglas enclosures were placed in the pond, each isolating about 700 liters of water and a 5-cm layer of sediment. Three of the enclosures were treated with 10 ppm sodium arsenate. The enclosures and the pond were monitored similarly to the microcosms.

The baseline stability, replicability, and persistence of the microcosms confirmed the results of the first experiment. Where comparisons could be made between the two experiments, the agreement was excellent, even though quite different communities were involved. Arsenic transport was found to be concentration-dependent. Effects of higher arsenic concentrations were seen on the primary production to

ecosystem respiration (P/R) ratio (Giddings and Eddlemon 1978), community structure, and the levels of some nutrients. Water chemistry in the control microcosms was very similar to that in the untreated pond enclosures and in the open pond. Effects of 10 ppm arsenic were similar in the microcosms and the enclosures, although arsenic transport differed somewhat between the two systems.

On the basis of our experience to this point, we drafted a tentative protocol for the use of microcosms to study contaminant fate and effects. The procedure for constructing a pond microcosm is simple. All microcosm components are collected from the pond on the day they are to be used. A 5-cm layer of sediment is placed in an aquarium which is then filled to the top with pond water. The macrophyte community is quickly drained and a weighed aliquot is placed in the microcosm. Except for distilled water to replace evaporation, and new pond water to replace the samples removed, no further additions to the microcosm are necessary.

When the protocol was drafted, our analysis of the first experiment had not yet revealed the effects of size on replicability and equilibration, and we felt that smaller microcosms would be better suited for screening purposes. Therefore we recommended 7-liter microcosms in the protocol. The protocol described a straightforward sampling program for assessing contaminant transport, and the P/R response was proposed as an index of ecosystem-level effects.

We tested the protocol using chromium as the test contaminant (Experiment 4). Sodium chromate was added to 7-liter microcosms in concentrations of 0.1, 0.25, and 1.0 ppm; this treatment was carried

out four times at two-week intervals. The transport of chromium was studied as in previous experiments, except that no radiotracer was used; instead, the results were based on stable chromium analysis. Primary production and ecosystem respiration were measured once or twice weekly.

The experiment proved successful in measuring the sediment-water exchange and bioaccumulation potential of chromium. However, P/R results were inconclusive because the microcosms were unstable and replication was poor. This was thought to be due to disturbances caused by sampling and P/R measurements, suggesting that 7-liter microcosms are too small for experiments of this type. The small size also made it difficult to collect sufficiently large samples for chromium analysis. Seventy-liter microcosms have been used in all subsequent experiments.

In connection with another project, two additional microcosm experiments on trace element transport were conducted. In one (Experiment 6), radiolabeled selenium (sodium selenate) was added to four microcosms containing fish (to be described below). Experiment 7 examined the transport of mercury in two microcosms without fish. Both of these experiments proceeded very smoothly (one was conducted by an undergraduate student).

We had originally planned to follow the trace metal experiments with a test of hexachlorobenzene (HCB), as a representative organic contaminant. It was later decided to use anthracene (a polycyclic aromatic hydrocarbon) instead of HCB, because the environmental transport and fate of this compound were already under intensive study by another research group within the Environmental Sciences Division. These

researchers (Dr. Stephen Herbes, Mr. George Southworth, and Ms. Linda Schwall) had measured sorption coefficients, octanol:water partitioning, volatilization, photolysis, hydrolysis, biodegradation, and bioaccumulation of anthracene under controlled laboratory conditions in one- or two-component systems (water, water plus sediment, or water plus organism). These background data would be useful for comparison with the interpretation of the microcosm results.

The microcosm experiment (Experiment 8) was begun in July 1977, with the addition of $0.5 \mu\text{Ci } [9-^{14}\text{C}]$ anthracene to each of two 6-month-old microcosms. The same general procedure was used as in the trace metal experiments. In this experiment, however, the chemical fate of the contaminant was studied in detail; extracts of water, sediment, and organisms were analyzed by thin-layer chromatography and radioautography to determine the amounts of anthracene and ^{14}C -labeled derivatives present. We found that anthracene sorbed rapidly to the sediment, and that anthracene remaining in the water was degraded by photolysis within 20 days. Anthracene persisted for the length of the experiment (84 days) in the sediment, where 17% of the original addition was recovered unchanged. These results were not inconsistent with the laboratory experiments, but direct comparisons were impossible without a unified model of transport and fate. Deriving a net degradation rate for anthracene from the microcosm data was relatively simple. Predicting a net degradation rate by combining single-process data in a mechanistic model is currently beyond our capability.

Explorations of the properties of pond microcosms continued through the final year of the project. A set of eight replicates was set up in

July 1977, following the routine procedure except that small fish (bluegill, fathead minnow, or mosquitofish) were added to six of the microcosms (Experiment 5). Water chemistry, biota, and ecosystem metabolism were monitored for two months, at which time the bluegill and mosquitofish microcosms were used for Experiment 6. The other microcosms were studied for an additional 6.5 months. One of the fathead minnow microcosms was very different from the other fish microcosms and the controls, with turbid water, low pH and dissolved oxygen, low productivity, and Elodea die-offs. It was discovered in early November that a large crayfish (about 10 cm) had been inadvertently included in this microcosm. None of the other microcosms exhibited similar destructive effects. The presence of fish did affect the benthic and periphyton communities, probably indirectly by eliminating snails; zooplankton were also sparse in the fish microcosms. Effects on nutrient concentrations were small, but gross primary production was over 50% higher in the bluegill and fathead minnow microcosms than in the others. Most of the fish survived without added food. Snails and crayfish have a significant impact on microcosm ecology. Our general conclusion was that fish are compatible with a stable and persistent microcosm community, although they may affect the community composition and metabolism.

Concurrently with the third year of the project, aquatic microcosm research was incorporated into an ongoing ORNL program dealing with the environmental effects of synthetic fuel production (sponsored by the Department of Energy). Our standard pond microcosms are now being used to study (a) the environmental transport and degradation of organic

compounds present in wastewater from coal conversion facilities, and (b) chronic effects of coal conversion effluents on pond ecosystems, with particular emphasis on the microbial community. In conjunction with this research, we are continuing to evaluate the microcosms in terms of ecological similarity to actual ponds, replicability, and repeatability.

3.2 SUMMARY OF RESULTS

3.2.1. Properties of Microcosms (J. M. Giddings)

3.2.1.1 Basic Ecological Characteristics of Pond Microcosms

Since the ecological properties of pond microcosms change with time, they are best described in terms of ecosystem development or succession. Odum (1969) defined ecosystem development by three parameters: (1) orderly changes in species structure and community processes; (2) modification of the physical environment by the community; and (3) culmination in a stabilized ecosystem. These events occur annually in pond ecosystems, and we have observed them in microcosms as well. Much of the following summary is drawn from Experiment 9 (see Appendix A), which was undertaken specifically to observe details of microcosm succession.

Species Structure

The pond community which is the source of our microcosm inocula is dominated by Elodea (pondweed) and Physa (snail) throughout the year, and consequently these organisms dominate the microcosms in terms of biomass and activity. Elodea masses are initially anchored by pushing

a few stems into the sediment, leaving most of the plants floating a few centimeters from the bottom. The floating stems produce dense roots within a few days, and after a week, most of the roots have begun to penetrate the sediment. The Elodea mass gradually spreads to cover 75 to 95% of the sediment surface, increasing in height until, after 3 to 6 months, nearly the entire microcosm volume is occupied. Physa, introduced as part of the Elodea community, begins depositing egg masses almost immediately, and newly hatched snails soon become abundant. Egg masses and snails of all sizes are always present in mature microcosms (except when fish are present - see 3.2.1.5).

Because the microcosms are spatially heterogeneous it is a practical impossibility to quantify the subdominant and minor components of the biota. To obtain replicable sampling units, we place artificial substrates (glass slides and cubes of laboratory sponge) in the microcosms, and remove them for examination after 2 to 14 days. The organisms colonizing these substrates are usually the same as those found in samples of surface sediment, detrital material, and periphyton. Filamentous blue-green algae (Oscillatoria, Anabaena), which often dominate the slide communities, form patches on the sediment surface within one or two weeks after a microcosm is constructed, and remain there unless disturbed. Other important components of the benthic flora are pennate diatoms and motile unicellular algae (Cryptomonas, Gymnodinium, Chlamydomonas, and Euglena). The algal community loses some rarer components during the first one or two weeks, but changes little after this time. From 20 to 30 algal species are normally found on a slide after three days in a microcosm, regardless of the age of the microcosm; 25 to 35 species are found on slides in the pond.

Animal populations in the microcosms are more changeable than the algae. Oligochaetes and dipteran larvae are usually abundant in the beginning, accompanied by Hydra, planaria, damselfly larvae, and water mites. Of these groups, only the oligochaetes persist in large numbers beyond the first few weeks. Protozoa, especially ciliates, appear early and become more numerous and diverse through time. Nematodes, rotifers, and gastrotrichs increase somewhat more slowly, and cladocera and copepods do not become abundant until the microcosms stabilize (about eight weeks). The animal communities which develop on glass slides in mature microcosms are very diverse; a typical example from a 10-week-old microcosm included more than 14 genera of protozoans (amoeboid, flagellate, and ciliate), plus several genera of rotifers, nematodes, gastrotrichs, and oligochaetes. A list of organisms that have been encountered in the microcosms is presented in Appendix B.

Community Metabolism

During Experiment 2, we began measuring net community primary production (P) and total ecosystem respiration (R) by the three-point dissolved oxygen method of McConnell (1962). In this procedure, the dissolved oxygen (D.O.) concentration is measured at the beginning of one dark period, the next light period, and the following dark period. The decline in D.O. during the dark period, corrected for diffusion between water and atmosphere, is a measure of total ecosystem respiration, and the rise in D.O. in the light represents net community primary production. In measurements made during the first year, we floated a sheet of Saran Wrap (PVC) on the surface of the water, eliminating diffusion and the need for a diffusion correction. Later, in a series of

measurements with the Saran Wrap alternately on and off, we found that the plastic caused a reduction in primary production, but did not affect respiration. From differences in nighttime oxygen loss rates with and without Saran Wrap, we were able to calculate diffusion rates as a function of D.O. saturation. The empirical relationship derived was:

$$D = 0.03 (S - 110) ,$$

where D = oxygen diffusion rate (ppm/12 hr) and S = % saturation. This relationship was used to correct for diffusion in subsequent experiments, and Saran Wrap was omitted. The diffusion rate was usually less than 10%, and rarely more than 15%, of the measured rate of change of oxygen concentration.

The most complete time-series of P and R measurements obtained so far came from Experiment 9 (Fig. 3.1). Primary production increased rapidly during the first 40 days, then leveled off abruptly, remaining within $\pm 10\%$ of a mean value from Day 50 until the termination of the experiment. Mean equilibrium values for the two replicates in this experiment were 8.9 and 7.5 ppm/12 hr. In other experiments, microcosms have shown equilibrium production rates between 4 and 8 ppm/12 hr. Assuming a photosynthetic quotient of 1.0, this range corresponds to 0.5 to 0.9 g C/m²/day, which is typical of ponds or shallow lakes (Odum 1969; Ruttner 1963). We have not collected enough data to determine the reasons for differences in P between experiments; variation in temperature or the nutrient content of the sediment may be responsible.

Respiration was initially higher than primary production. The respiration rate increased gradually, reaching a plateau after about

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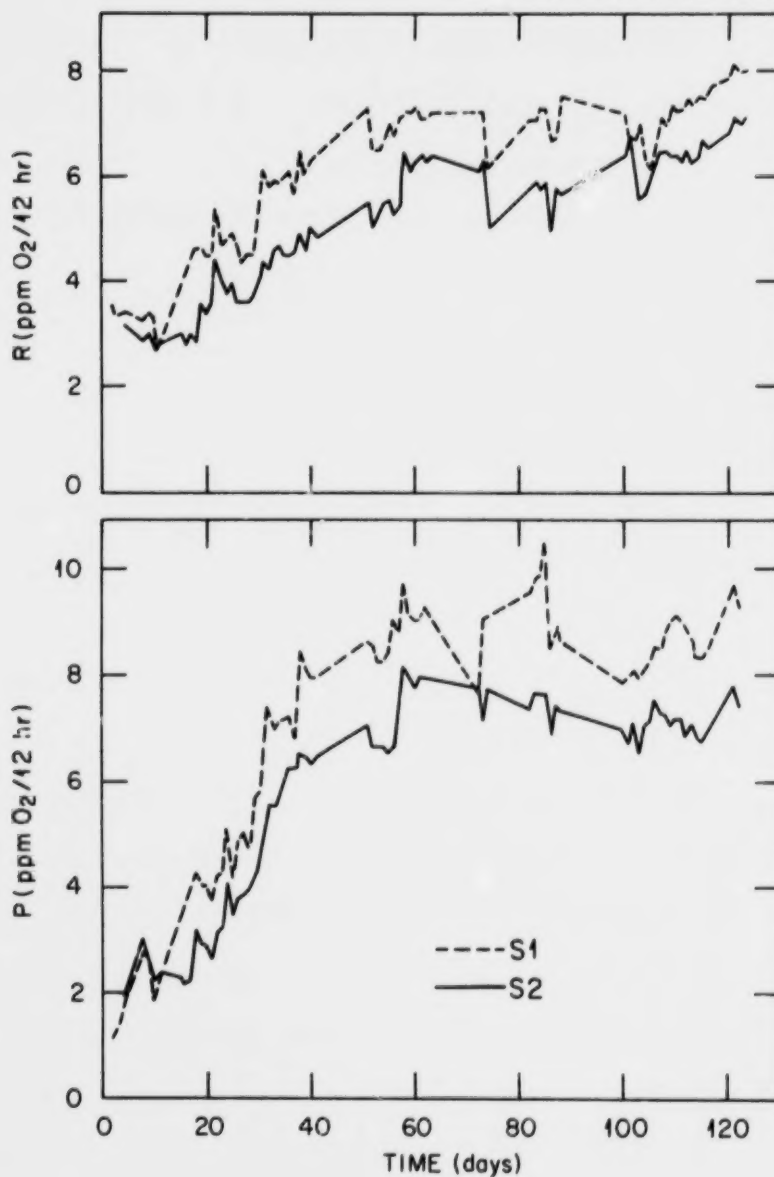


Figure 3.1. Production and respiration rates in microcosms from Experiment 9. P = dissolved oxygen increase during the light period; R = dissolved oxygen decrease during the dark period. Both rates are corrected for diffusion, as explained in the text. S1 and S2 are the two replicates.

50 days (Fig. 3.1). The mean equilibrium values for the two replicates shown were 7.3 and 6.1 ppm/12 hr.

Figure 3.2 shows production/respiration ratios for the two microcosms depicted in Fig. 3.1, plus data from two other experiments. The ratio was well below 1 at the beginning of the experiment but rose rapidly. Values of P/R in equilibrated microcosms fell in the range 1.0 to 1.4. It is worth noting that the P/R ratio, an integrative measure of ecosystem function, is more consistent between experiments, and between replicates within an experiment, than either P or R alone.

A P/R ratio consistently above 1 implies that more organic matter is being formed than is being remineralized in the system. The excess production accumulates in the microcosm as living biomass, detrital particles, and dissolved organic matter. A steady increase in the latter has been observed in every experiment where analyses were made (Fig. 3.3). Accumulation of particulate and dissolved organic matter during the growing season occurs in ponds and shallow lakes (Allen 1969). The concentration of dissolved organic carbon is the only chemical parameter we have measured which continues to change after the first 60 days in a microcosm.

Modification of the Physical Environment

During the course of ecosystem development in pond microcosms, water chemistry and sediment structure are progressively altered by the activities of the biota. As a result of rapid plant growth during the first two months, dissolved oxygen and pH steadily increase, while concentrations of inorganic nitrogen, phosphorus, and carbon decline.

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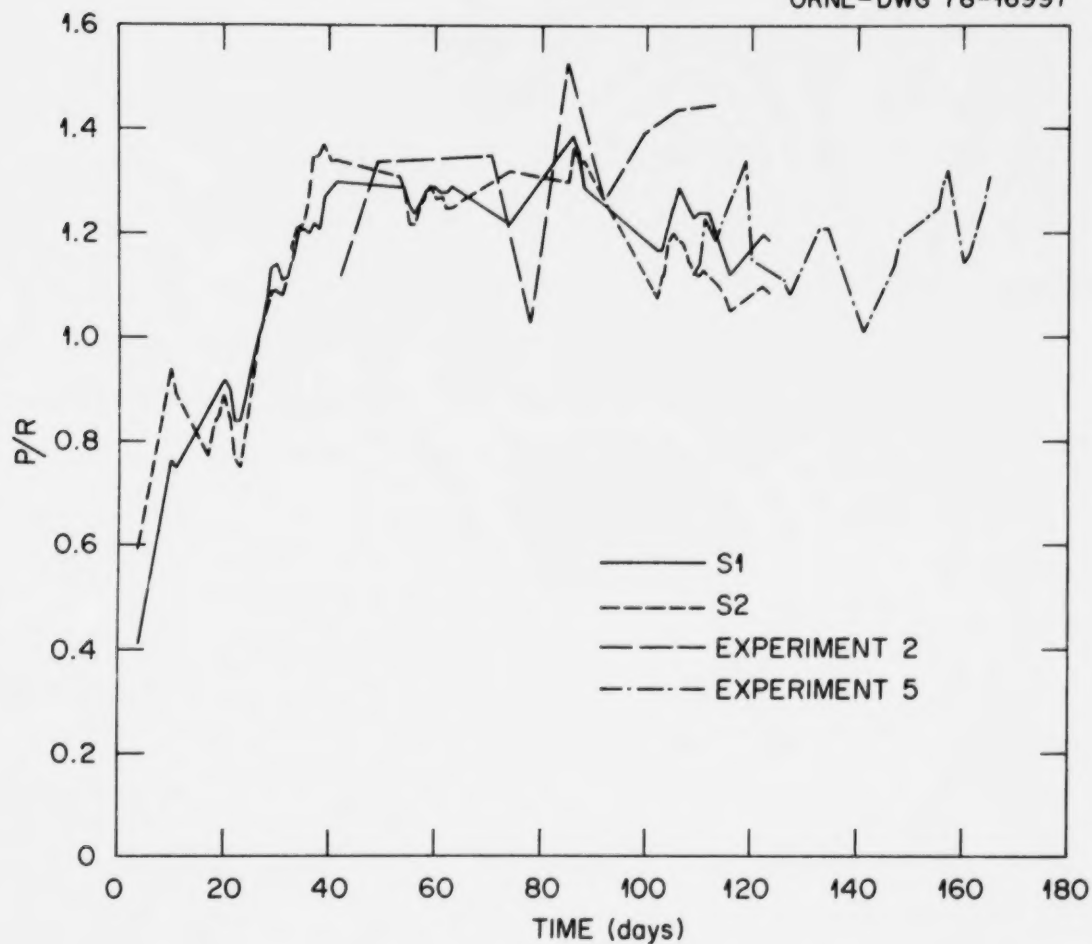


Figure 3.2. Production/respiration ratios from three microcosm experiments. S1 and S2 are the two replicates in Experiment 9. Values from Experiment 2 are averages from the three control microcosms. The Experiment 5 line represents control microcosm C1. Data for Experiment 2 are results of weekly measurements; all others are 3-day moving averages.

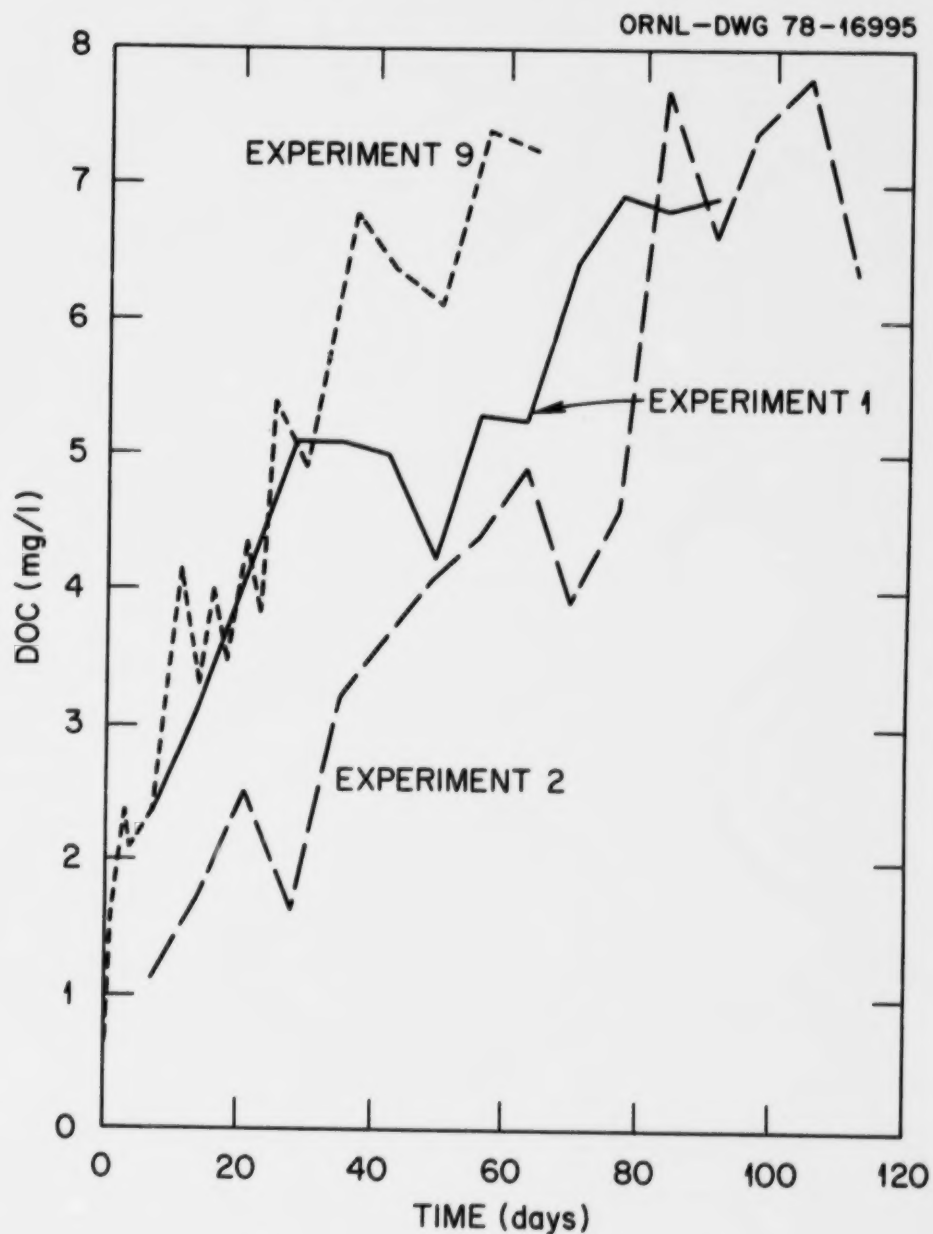


Figure 3.3. Dissolved organic carbon in microcosm water from Experiment 1 (mean of three 70-liter sediment microcosms), Experiment 2 (mean of three control microcosms), and Experiment 9 (mean of two microcosms).

Changes in mid-afternoon D.O. and pH in Experiment 2 control microcosms are shown in Fig. 3.4. At the beginning of the experiment, respiration exceeded photosynthesis, which would have resulted in a drop in D.O.; this was counteracted by diffusion of oxygen from the atmosphere into the water, because D.O. levels were below saturation for most of the day. Later, when P/R was greater than 1, the D.O. concentrations were above saturation, and the excess oxygen produced diffused out of the water into the air. The relatively constant D.O. concentrations (measured at the same time each day) in mature microcosms reflect a balance between photosynthesis, respiration, and diffusion.

Changes in pH during Experiment 2 closely paralleled changes in D.O. (Fig. 3.4). The increase over the first seven weeks was accompanied by a decline in dissolved inorganic carbon from 2.4 to 0.4 meq/liter. Most of the inorganic carbon was probably incorporated into biomass, but some was seen to precipitate (presumably as CaCO_3) on the surfaces of macrophyte leaves as the pH rose.

Figure 3.5 shows the concentrations of total P, $\text{NH}_4\text{-N}$, and $\text{NO}_3\text{-N}$ in the water of microcosms from Experiment 9. Phosphate-P concentrations are not included in this figure because they were nearly always below 0.001 mg/liter. Total P varied between 0.004 and 0.02 mg/liter, with no obvious trends over the first 64 days. Nitrate concentrations declined during the first few days, rose to a secondary maximum at about 15 days, and then declined to low levels for the rest of the experiment. Ammonium behaved similarly, except that the secondary peak was spread over a period of about two weeks. After about 30 days, inorganic N was scarce in the microcosm water; nevertheless, plant biomass and primary production continued to increase for several more weeks (Fig. 3.1).

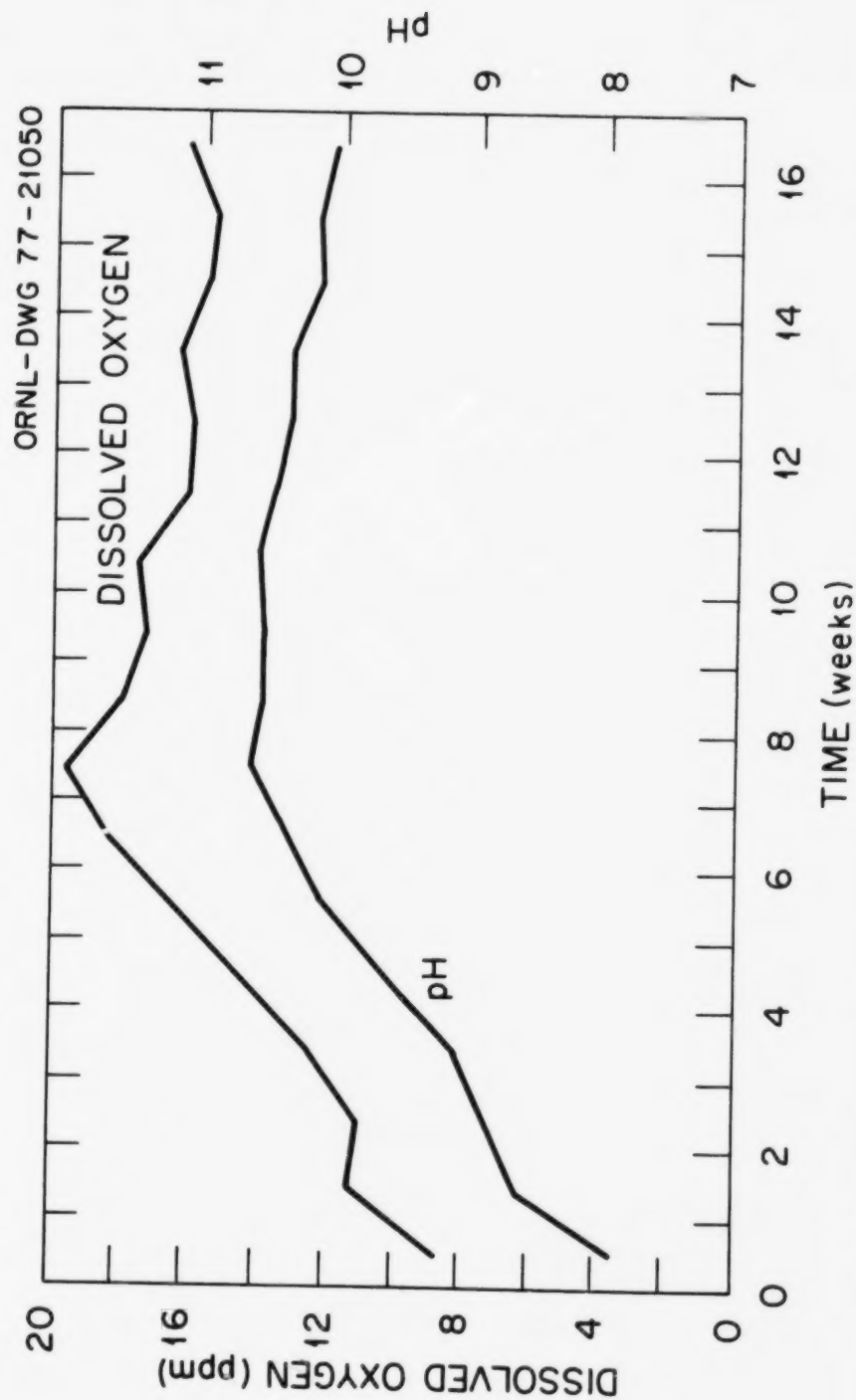


Figure 3.4. Dissolved oxygen and pH in microcosm water, Experiment 2. Each line shows the weekly mean for three replicates, based on 1 to 5 measurements per week.

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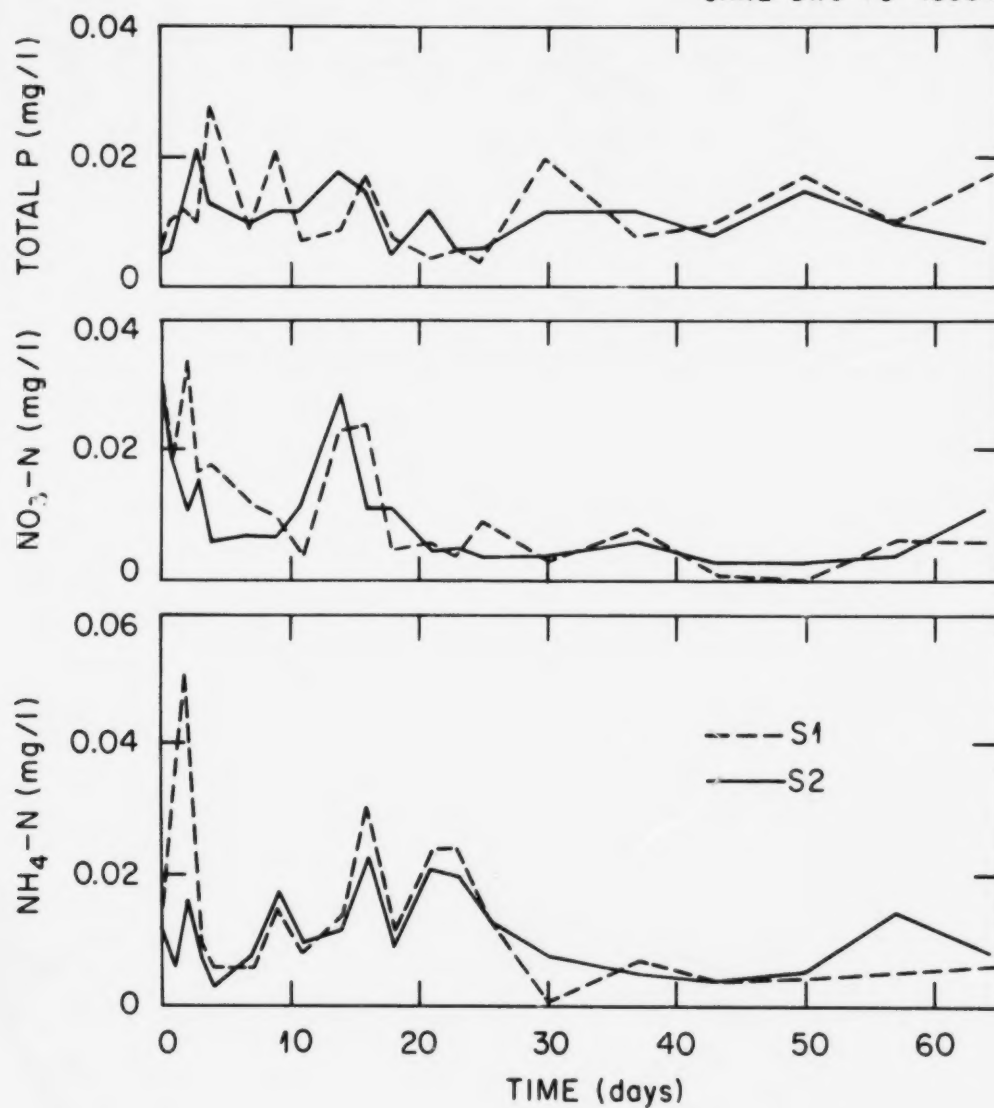


Figure 3.5. Nitrate, ammonium, and total phosphorus concentrations in microcosm water, Experiment 9. S1 and S2 are the two replicates.

A better picture of plant-nutrient relationships in the microcosms was obtained by analyzing interstitial water extracted from the sediment. These samples were taken by means of Amicon hollow fibers buried 2 cm below the sediment surface during construction of the microcosms. The data are shown in Fig. 3.6. Total P was even lower in the interstitial water than in the overlying water. Interstitial nitrate concentrations were initially higher than in the overlying water, but were reduced to undetectable levels by Day 9. The interstitial water contained high concentrations of $\text{NH}_4\text{-N}$ on Day 2, and the concentrations increased dramatically to a maximum of 1.4 to 1.9 mg/liter on Day 11. The source of this ammonium may have been bacterial decomposition of organic matter or excretion by benthic animals. After Day 11, interstitial ammonium concentrations declined gradually, becoming undetectable on Day 50, at which time the net primary production rate stopped increasing (Fig. 3.1).

The observation that inorganic nutrient pools are very low in mature microcosms implies that nutrient uptake by primary producers (and possibly bacteria) is equal to nutrient regeneration by animals and decomposers. This situation seems inconsistent with P/R ratios greater than 1. If oxygen production in the microcosms is greater than oxygen consumption, it might be expected that nutrient uptake would be greater than nutrient regeneration. There are several possible explanations:

- (1) The P/R estimates may be in error due to retention of gas in the internal spaces of the macrophytes (Wetzel 1964). This factor would

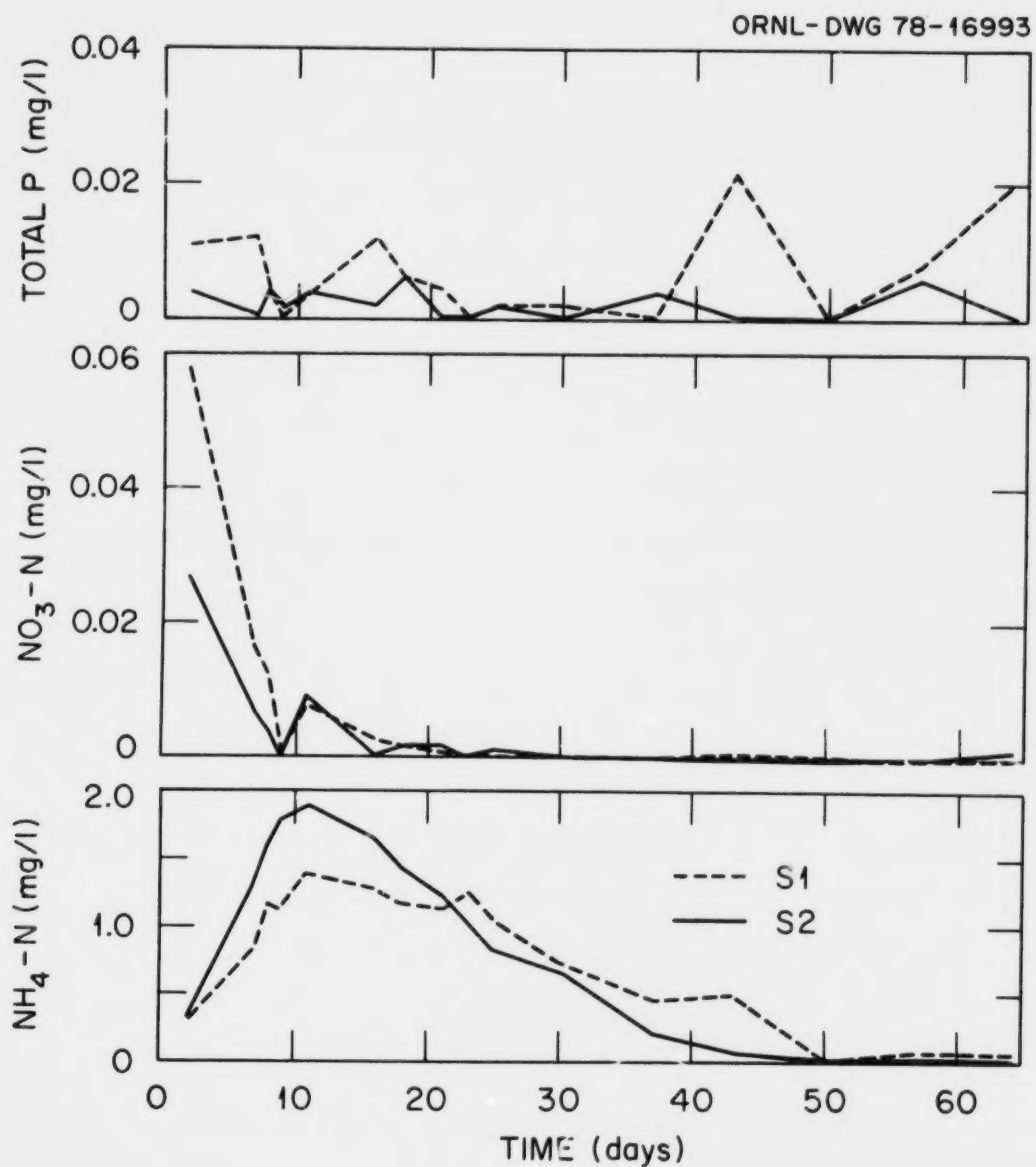


Figure 3.6. Nitrate, ammonium, and total phosphorus concentrations in microcosm interstitial water, Experiment 9. S1 and S2 are the two replicates.

lead to underestimates of both P and R, although its effect on P/R calculations would probably be small once equilibrium was reached. Overestimation of diffusion could also introduce a systematic error into P/R calculations, but long-term constancy of D.O. maxima (Fig. 3.4) and minima would be compatible with $P/R = 1$ only if diffusion were zero (which is unlikely since D.O. concentrations in the microcosms are usually well above saturation).

(2) The nutrients incorporated into excess organic production may be drawn from reservoirs in the sediment other than interstitial water. For example, Elodea roots may be capable of removing phosphate ions adsorbed to sediment particles.

(3) Nutrients may be regenerated from organic matter faster than carbon. Thus, more nutrients would become available per molecule of oxygen consumed than would be assimilated per molecule of oxygen produced. This has been observed in studies of detrital decomposition in streams (Kaushik and Hynes 1971, Triska et al. 1975).

(4) Nutrients may be regenerated by anaerobic processes in the deeper sediment.

Which of these explanations is the correct one cannot be determined from our data. Perhaps each of these factors contributes to the situation we observed. In any case, it is certain that a considerable amount of nutrient recycling occurs in mature microcosms. This subject deserves greater study.

Progressive stratification of the sediment was mentioned at the beginning of this section as another indication of biological modification of the physical environment. The sediment is nearly homogeneous

when a microcosm is first assembled, and vertical differentiation becomes apparent within a few days. This phenomenon has not been closely examined in our microcosms, but the development of three distinct zones can be seen even by casual visual inspection. The upper zone, 2 to 3 cm thick in a mature microcosm, consists of loose, light-brown material and is heavily worked by oligochaetes, snails, and other benthic animals. Beneath this zone is a 1- to 2-cm rust-colored layer, below which the sediment is dark brown, compact, and probably anaerobic (the presence of H_2S is noticeable). The structuring of the sediment results from the combined activities of bacteria, benthic fauna, and primary producers. The existence of microzones within the sediment is undoubtedly a significant factor in the ecology of the microcosms, especially nutrient cycling, and also influences the fate of added contaminants.

Equilibration

In the first stage of microcosm development, nutrient flux is mainly from inorganic reservoirs into biomass; this is a one-way flux, and the nutrient cycle is said to be open. The second, or equilibrium stage, begins when nutrient reservoirs are exhausted (Fig. 3.6) and the nutrient cycle becomes closed. This progressive closing of the nutrient cycle is a general feature of ecosystem development (Odum 1969). In a mature system, nutrient concentrations and flux rates are under biological control. Stability (in the sense of internal constancy) is maintained by a complex web of interactions between organisms (Reichle et al. 1975, O'Neill 1976). Relative constancy of conditions in mature

microcosms has been described above for primary production and ecosystem respiration (Fig. 3.1), the P/R ratio (Fig. 3.2), pH and dissolved oxygen (Fig. 3.4), and nutrient concentrations (Figs. 3.5 and 3.6). Our microcosms are stable because they are assembled in such a way that many of the biotic interactions which confer homeostasis are preserved. The accumulation of dissolved organic matter (Fig. 3.3), and probably also of particulate organic detritus, indicates that organic production and remineralization are not completely balanced, even in mature microcosms, and this may eventually lead to a collapse of stability. However, we have maintained microcosms for as long as nine months with no sign of weakened stability, and we expect that experiments lasting more than a year are possible. This property makes the microcosms suitable for long-term studies of contaminant degradation, chronic effects, and patterns of recovery from pollution. Model food chain microcosms (Metcalf et al. 1971) do not reach an equilibrium, and experiments must be terminated after about 30 days.

3.2.1.2 Comparison of Microcosms with an Actual Pond

Experiments 2 and 3 were conducted during the summer of 1976 to compare the ecological characteristics of pond microcosms with conditions in the pond itself and with conditions within enclosures in the pond. A shallow pond (mean depth approximately 1 m), formerly used for holding fish, was selected. This pond has a rock bottom overlain by 5 to 10 cm of fine sediment and covered with a dense bed of Elodea. Twelve 70-liter microcosms were established using sediment and Elodea

from the pond (Experiment 2). Six Plexiglas enclosures, each 0.6 x 1.2 m and extending from the sediment to above the water level, were then placed in the pond (Experiment 3). After 10 weeks, sodium arsenate was added to nine of the microcosms (three replicates each at 0.066, 11.5, and 143 ppm) and to three of the enclosures (9.2 ppm). Six weeks later, concentrations of nutrients and major ions were measured in the microcosms, the enclosures, and the pond.

The final concentrations of the major ions and nutrients in the pond, the control enclosures, and the treated enclosures are compared with the control and the 11.5-ppm microcosms in Table 3.1. Differences between control microcosms and the pond were significant ($p < 0.05$) only for Na, NH_4 , and total P. There were no significant differences between control microcosms and control enclosures. In both the microcosms and the enclosures, arsenic concentrations in the 10-ppm range caused rapid disintegration of the Elodea community and replacement by Spirogyra, accompanied by temporary declines in pH and D.O. The effects of arsenic treatment on water chemistry (elevated conductivity, alkalinity, and Na, K, and Ca concentrations) were similar in the enclosures and in the microcosms, with significant differences found only for Na, NH_4 , and PO_4 .

3.2.1.3 Replicability

The replicability of microcosms is one of the principal advantages of microcosms over field studies. The subject of microcosm replicability (similarity between replicates in a single experiment) has been specifically addressed by Abbott (1966) and by Isensee (1976). Both

Table 3.1. Comparison of water chemistry in microcosms, pond enclosures, and a pond^a

	Microcosms		Pond enclosures		Pond
	Controls	11.5 ppm	Controls	9.2 ppm	
Na	0.03 (0.02)	8.25 (1.00)	1.01 (0.71)	5.89 (0.28)	0.87
K	0.10 (0.0)	1.87 (1.86)	0.17 (0.28)	0.60 (0.11)	0.23
Ca	11.23 (0.47)	19.13 (8.20)	13.9 (3.2)	19.9 (4.1)	11.9
Mg	5.13 (0.67)	6.17 (1.17)	4.4 (0.3)	4.7 (0.5)	3.4
Cl	0.30 (0.04)	1.36 (0.63)	1.25 (0.86)	2.05 (0.44)	0.40
SO ₄	3.18 (2.50)	2.94 (0.47)	1.59 (0.62)	2.34 (0.87)	3.44
NO ₃	0.006 (0.005)	0.007 (0.002)	0.012 (0.007)	0.008 (0.002)	0.006
NH ₄	0.007 (0.002)	0.010 (0.004)	0.011 (0.002)	0.030 (0.011)	0.019
PO ₄	0.002 (0.001)	0.003 (0.001)	0.004 (0.002)	0.011 (0.003)	0.002
Total P	0.006 (0.003)	0.029 (0.017)	0.015 (0.010)	0.013 (0.004)	0.014
DOC	7.18 (1.39)	6.19 (1.26)	6.97 (2.15)	7.19 (0.62)	3.75
Alkalinity	49 (4.4)	117 (24)	52 (10)	75 (13)	43

^aAll concentrations in ppm; standard deviations in parentheses.

authors concluded that coefficients of variation (C.V.) of most microcosm measurements are about 10 to 30%, comparable to those of typical laboratory or greenhouse experiments. Our results support this conclusion.

Table 3.2 summarizes the C.V. in thirteen chemical parameters as determined in Experiments 1 and 2. The values in this table were derived by calculating the C.V. among 3, 6, or 12 replicates on each sampling date, and averaging across 1 to 12 dates within a single experiment. Coefficients of variation averaged less than 20% for total dissolved solids, alkalinity, Ca, Mg, and Cl. Sodium probably also belonged in this group--the absolute concentrations in the final samples from Experiment 2 were very low (0.01-0.05 mg/liter), so the 66% C.V. may be misleading. The same was true of K concentrations, which were below 0.01 mg/liter in mature microcosms. Concentrations of SO_4 , dissolved organic carbon (DOC), and dissolved nutrients were more variable. The variability of nutrients is not surprising, since these small, rapidly turning-over reservoirs are highly variable even in a single microcosm (or other aquatic ecosystem) over short time periods.

Conductivity, pH, and dissolved oxygen, the most frequently measured parameters in our experiments, have low coefficients of variation. Data from the 10-week pretreatment period of Experiment 2, when 12 replicates existed, are presented in Fig. 3.7. It can be seen that the individual microcosms diverged somewhat over the first four weeks, but then converged again. Convergence was also evident for PO_4 , total P, and DOC, but not for NO_3 or NH_4 (Fig. 3.8). The period of divergence reflects differences in rates of microcosm succession, while the

Table 3.2. Coefficients of variation: Microcosm water chemistry

Variable	Experiment 1 ^a	Experiment 2 ^b
NH ₄	51 (11) ^c	49 (9)
NO ₃	73 (11)	51 (9)
PO ₄	63 (11)	50 (9)
Total P	41 (11)	39 (9)
Na	7 (12)	66 (1)
Ca	9 (12)	9 (1)
Mg	5 (12)	18 (1)
Cl	10 (12)	12 (1)
SO ₄	63 (12)	47 (1)
K	86 (12)	0 (1)
HCO ₃	6 (7)	9 (9)
TDS	6 (12)	-
DOC	-	35 (9)

^aC.V. calculated for three replicates.

^bC.V. calculated for six replicates for Na, Ca, Mg, Cl, SO₄, K; other variables, 12 replicates.

^cValues expressed as percentages; the number of weekly C.V. averaged is given in parentheses.

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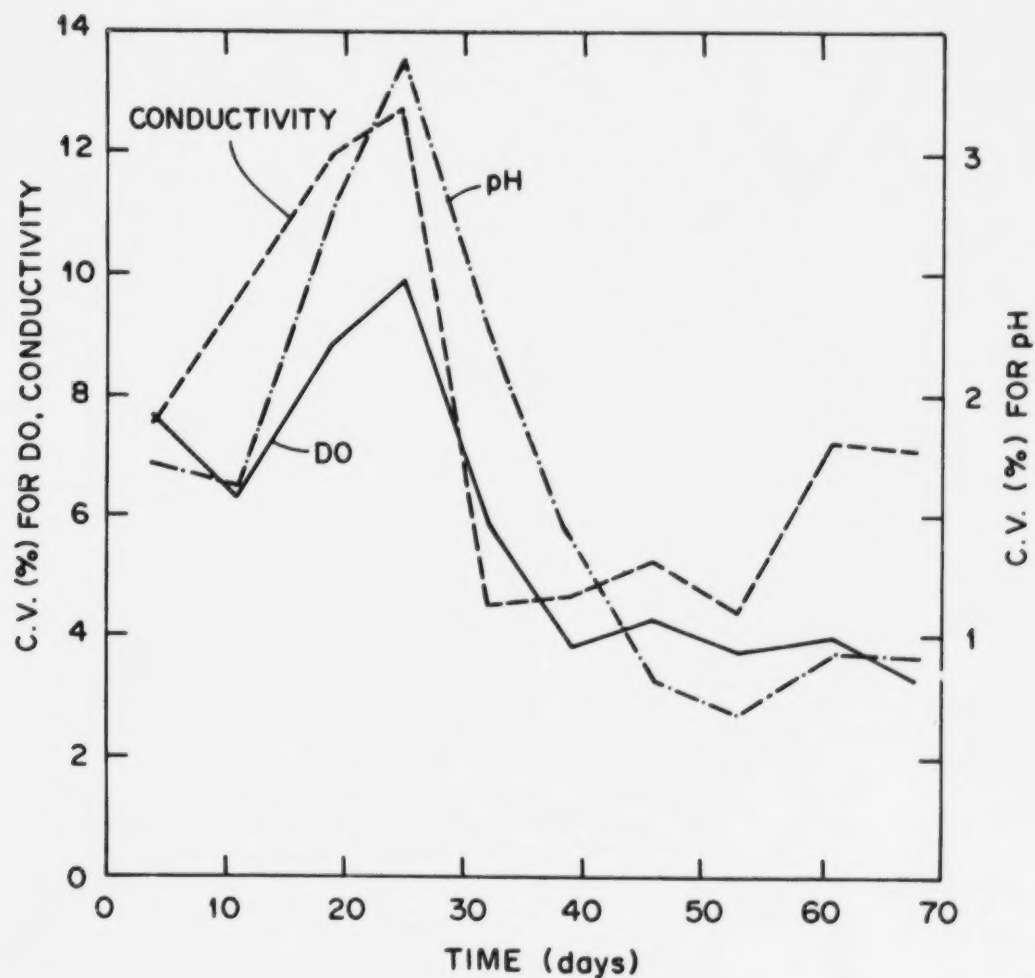


Figure 3.7. Coefficients of variation of conductivity, dissolved oxygen, and pH, Experiment 2. C.V. expressed as percentages for twelve replicate microcosms.

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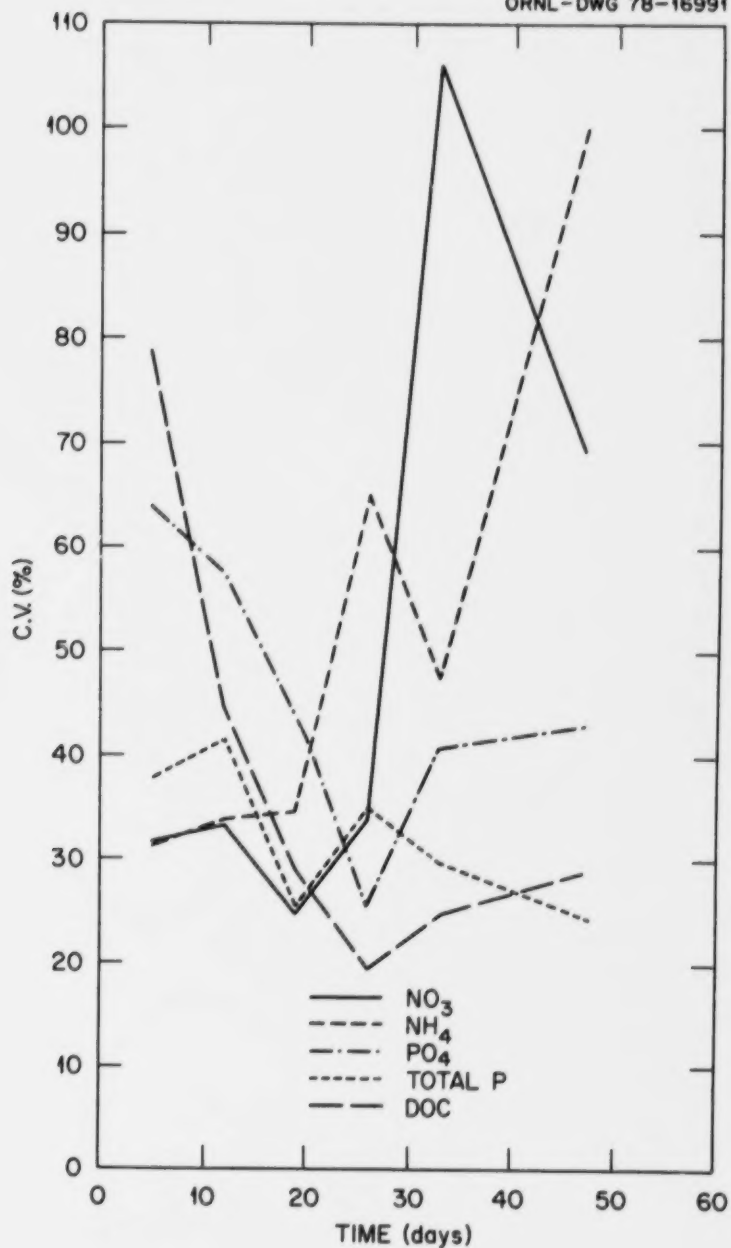


Figure 3.8. Coefficients of variation of nitrate, ammonium, phosphate, total phosphorus, and dissolved organic carbon, Experiment 2. C.V. expressed as percentages for twelve replicate microcosms.

subsequent convergence implies that the limits to succession are similar among replicate microcosms. It would be interesting to see whether disturbances (chemical or otherwise) imposed at the beginning of succession might increase the divergence, or prevent later convergence, by interfering with the self-regulating capacity of the microcosm.

It has been suggested (Dudzik et al., 1979) that trace contaminant experiments in pelagic microcosms should focus on the initial developmental phase. In pond microcosms, this strategy would be expected to increase the variability of data.

Data from Experiments 2 and 4 permit an assessment of the variability of metabolic measurements in microcosms. In both studies, rates of primary production and total community respiration were measured repeatedly by the diurnal oxygen technique (McConnell 1962) in a set of replicate microcosms. In Experiment 2, primary production and respiration were measured in 12 70-liter microcosms on three occasions during the pretreatment period. The average coefficients of variation among microcosms were 15% for production, 11% for respiration, and 12% for the P/R ratio. In Experiment 4, eight 7-liter microcosms were monitored 19 times in 11 weeks. Coefficients of variation were 24% (P), 21% (R), and 18% (P/R). These results are in the same range as those of previous investigators (Table 3.3).

Coefficients of variation for concentrations of several test contaminants in microcosm components are shown in Table 3.4. Except in the case of snails, all of these values are below 30% and the average is 19%. (It should be noted that these data are based on analyses made at the termination of each experiment.) For comparison, Table 3.4 also

Table 3.3. Coefficients of variation: Ecosystem metabolism

	Coefficient of variation (%)		
	Production	Respiration	P/R
Experiment 2	15	11	12
Experiment 4	24	21	18
Beyers 1963	15	14	7
McIntire et al. 1964	14	33	21
Abbott 1966	13	7	8

Table 3.4. Coefficients of variation: Contaminant concentrations

	Water	Sediment	Algae	<u>Elodea</u>	<u>Physsa</u>	n ^a
Arsenic						
Experiment 1	28 ^b	27	29	-	33	3
Experiment 2 ^c	26	6	21	-	51	3
Chromium						
Low dose	6	5	22	-	10	2
High dose	6	12	9	-	35	2
Selenium						
Bluegill	8	1	-	11	-	2
Mosquitofish	15	9	-	39	-	2
Mercury	26	23	-	10	8	2
Anthracene	2	12	19	6	43	2
AVERAGE	15	12	20	17	30	
(Range)	(2-28)	(5-27)	(9-29)	(6-39)	(8-51)	
Isensee 1976						
AVERAGE ^d	5	-	20	-	32	
(Range)	(1-11)	-	(1-59)	-	(12-59)	

^aNumber of replicate microcosms.

^bValues expressed as percentages.

^c0.066-ppm treatment.

^dFor 10 contaminants tested.

summarizes Isensee's data from 10 experiments with insecticides in modified Metcalf microcosms (Isensee 1976). The agreement between the two types of microcosms is strong, suggesting that system complexity per se does not affect the variability of microcosm results.

3.2.1.4 Repeatability

One important rationale for defined (or gnotobiotic) microcosms, such as the Metcalf system, is that an experiment can be repeated at any time by reassembling microcosms from the same stock cultures. Since our microcosms are constructed from natural communities, seasonal and spatial variability within the pond ecosystem might lead to irreproducibility of experiments. This situation would be a drawback in screening work, although the ability to reflect natural variability could be considered an asset when realistic simulation is desired.

A high degree of consistency between experiments has already been implied in the discussion of ecosystem development (Section 3.2.1.1). Differences in the species composition of inocula are usually minor, but even when irregular inocula are used (such as in Experiment 8, where Elodea were not added directly but allowed to grow from propagules in the sediment), typical Elodea-Physa communities develop in mature microcosms. In other words, convergence occurs not only within an experiment (Section 3.2.1.3) but also among experiments. Some examples of inter-experiment comparisons may be seen in Figs. 3.2 and 3.3.

From the standpoint of contaminant research, reproducibility of transport data is perhaps more critical than reproducibility of the test system. We have not normally repeated transport experiments, but

a comparison is possible between the 70-liter sediment microcosms of Experiment 1 and the 0.066 mg/liter arsenic treatment of Experiment 2. The time course of loss of arsenic from the water was nearly identical in the two experiments (Fig. 3.9). Final arsenic concentrations in water, sediment, and snails were not significantly different among experiments, whereas algae accumulated significantly more arsenic in the first experiment (Table 3.5). In view of the similarity between results of these two experiments, it is worth enumerating the differences in procedure followed in each case. Experiment 1 was begun in February, Experiment 2 in July. The sediment used in Experiment 1 was collected from two local reservoirs and consisted mainly of terrigenous silt (4.9% organic matter), while that in Experiment 2 came from a small fish pond and was autochthonous in origin (6.8% organic matter). Experiment 1 sediment was oven-dried, ground with a mortar and pestle, and homogenized before use, while Experiment 2 sediment was transferred directly from the pond to the microcosms. The inoculum for Experiment 1 was a filamentous algal community from an outdoor concrete tank, whereas an Elodea community from the fish pond was used in Experiment 2. In Experiment 1, adult snails were counted and added to the microcosms directly; in Experiment 2, they were included as a natural component of the inoculum. Finally, water temperature was 17 to 19°C in Experiment 1, and 19 to 21°C in Experiment 2. These two sets of microcosms, containing completely different communities collected at different seasons from different places, gave very similar results on arsenic transport. We are, therefore, encouraged to believe that results from microcosms constructed according to our standard procedure will not be greatly

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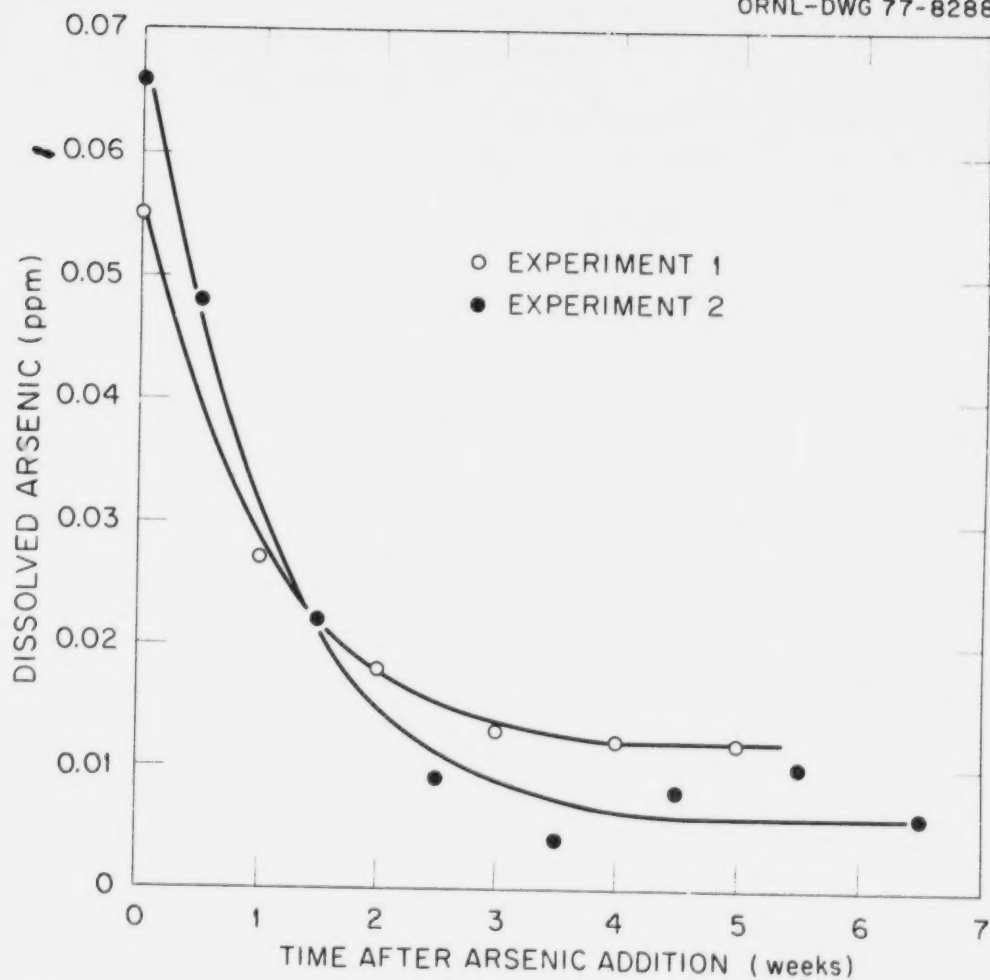


Figure 3.9. Dissolved arsenic concentrations, Experiments 1 and 2. Points are weekly averages for three replicate 70-liter microcosms.

Table 3.5. Arsenic concentrations in microcosm components - Comparison of Experiment 1 and Experiment 2^a

	Experiment 1 (ppm)	Experiment 2 (ppm)
Water	0.010 $\pm$ 0.003	0.007 $\pm$ 0.002
Sediment	0.529 $\pm$ 0.144	0.522 $\pm$ 0.032
Algae	15.24 $\pm$ 4.38	2.14 $\pm$ 0.45
Snails	2.41 $\pm$ 0.78	3.22 $\pm$ 1.63

^aMean $\pm$ standard deviation, three replicate microcosms.

dependent on season or source of inoculum. In spite of this convergence between experiments, however, the most reliable simulations of a given aquatic environment would be expected from microcosms whose components come from that environment.

3.2.1.5. Effects of Variations in Experimental Condition and Microcosm Design

Microcosm Size

In Experiment 1, we compared 7-liter and 70-liter microcosms in terms of ecological properties and arsenic transport. We found no significant effect of size on equilibrium dissolved oxygen (Fig. 3.10), pH (Fig. 3.11), or nutrient levels (except Fe, Table 3.6), nor on the concentrations of most ions (Table 3.7). Final algal biomass and chlorophyll-a per cm^2 were the same for both sizes (Figs. 3.12 and 3.13). Patterns of arsenic transport were similar in the two sizes, but the rate of movement of arsenic from water to sediment was higher in the small systems (Fig. 3.14). This in turn resulted in higher concentrations in snails and lower concentrations in algae in the smaller microcosms (Table 3.8). Effects of size in this experiment were minor, however, compared to the effects of substrate type (see below).

Baseline stability and equilibration times were related to microcosm size. Coefficients of variation for D.O. and pH in the sediment microcosms were calculated for overlapping two-week intervals, with the results shown in Figs. 3.15 and 3.16. Coefficients of variation declined over time for both variables, indicating progressive stabilization of the microcosms. The declines in coefficients of variation occurred sooner in the 70-liter microcosms than those in the 7-liter microcosms; that is, equilibration was more rapid in the larger systems.

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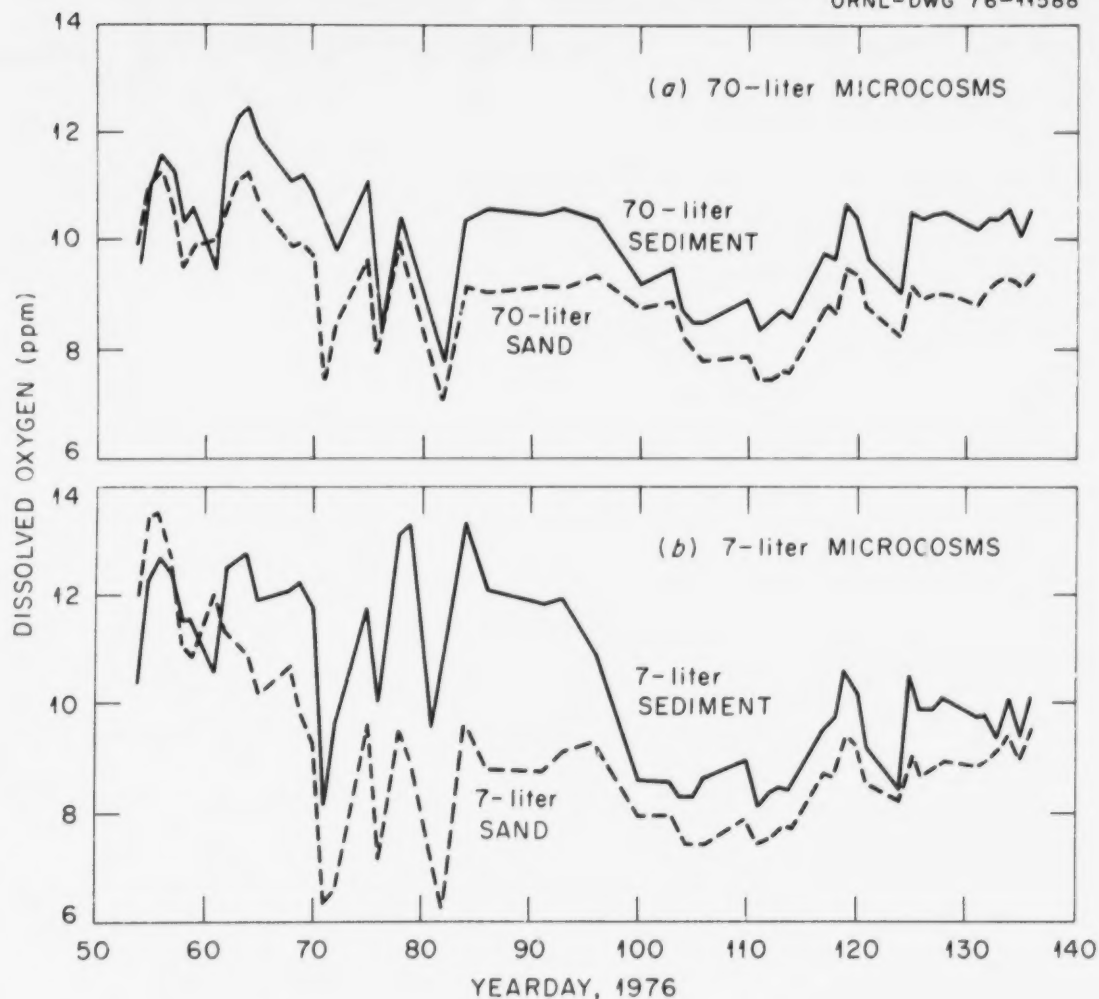


Figure 3.10. Dissolved oxygen concentrations, Experiment 1. Each line represents the mean of three replicate microcosms.

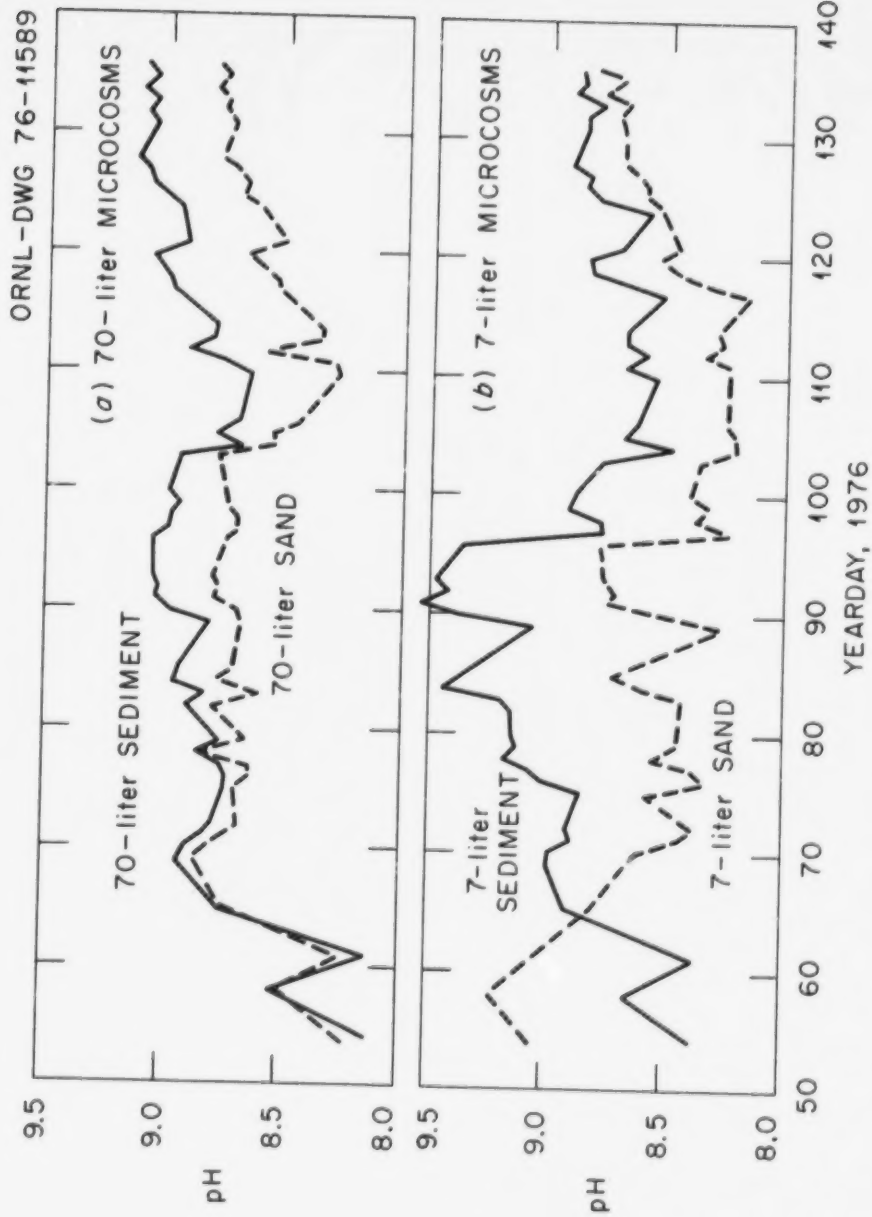


Figure 3.11. pH, Experiment 1. Each line represents the mean of three replicate microcosms.

Table 3.6. Mean nutrient concentrations: Experiment 1^a

	70-liter sediment	70-liter sand	7-liter sediment	7-liter sand
Fe ^{b,c}	0.027	0.000	0.145	0.000
NO ₃ -N ^b	0.058	0.014	0.085	0.014
NH ₄ -N	0.012	0.013	0.079	0.016
PO ₄ -P	0.004	0.008	0.013	0.005
Total P	0.021	0.017	0.029	0.018

^aAll values are in ppm. Each is a mean for three microcosms, 13 weekly determinations.

^bSignificant difference between sand and sediment microcosms ($p < 0.05$).

^cSignificant difference between 70-liter and 7-liter microcosms ($p < 0.05$).

Source: Giddings and Eddlemon 1977.

Table 3.7. Summary of water chemistry data: Experiment 1^a

	Spring water ^b	70-liter sediment	70-liter sand	7-liter sediment	7-liter sand
Na ^c	0.42	1.06	0.90	1.39	1.47
K ^c	0.53	0.51	0.46	0.95	1.55
Ca ^d	18.8	22.52	17.34	22.39	19.70
Mg ^d	9.4	10.76	8.69	10.46	10.30
Cl ^c	1.10	1.72	1.68	3.10	3.26
SO ₄	2.25	3.78	4.38	3.85	7.80
HCO ₃	84.0	97.8	75.9	95.8	85.2
TDS ^d	117.0	138.0	109.0	138.0	129.0

^aAll values are ppm. HCO₃ expressed as equivalent CaCO₃. Means of three replicates, 13 weekly determinations.

^bSpring water values are the result of a single analysis at the beginning of the experiment.

^cSignificant difference between 70- and 7-liter microcosms ($p < 0.05$).

^dSignificant difference between sand and sediment microcosms ($p < 0.05$).

Source: Giddings and Eddlemon 1977.

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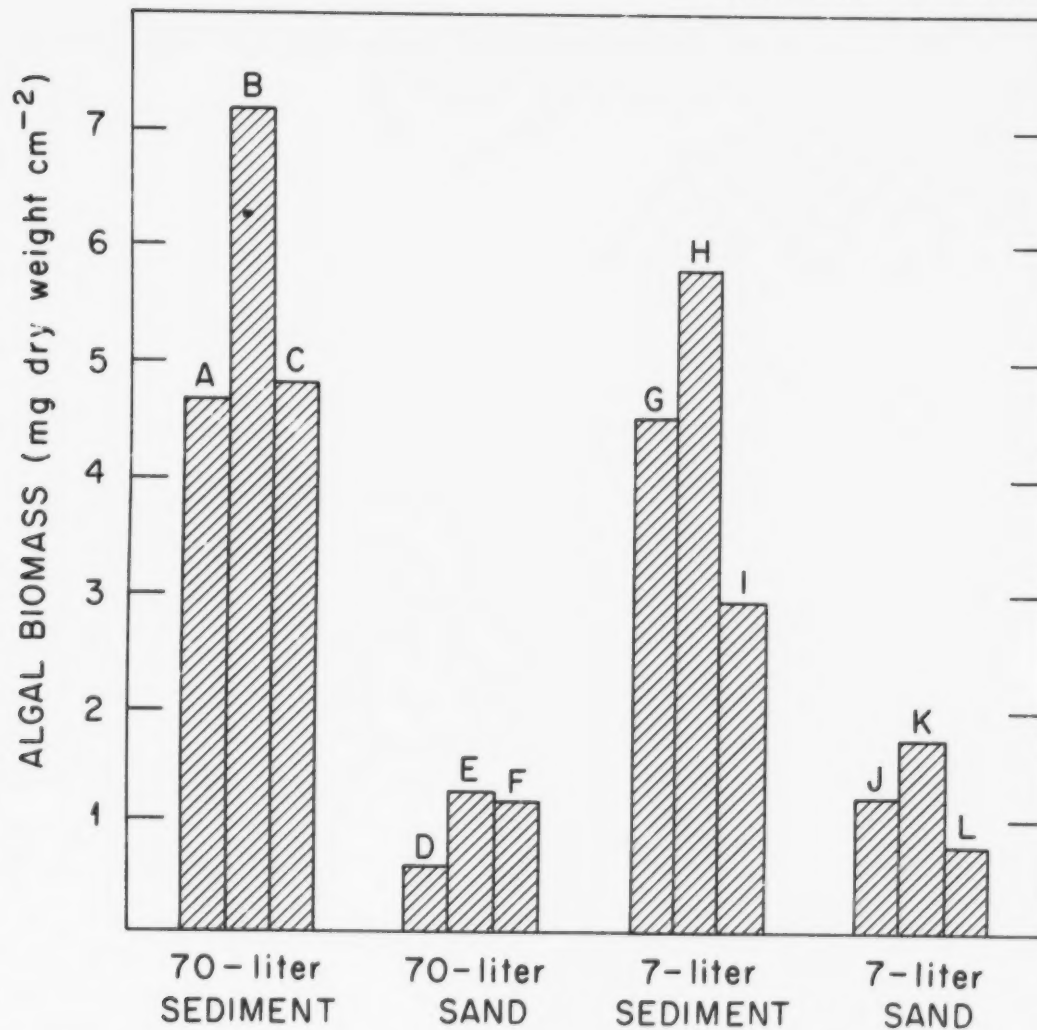


Figure 3.12. Final algal biomass, Experiment 1. Bars represent replicates identified by letter.

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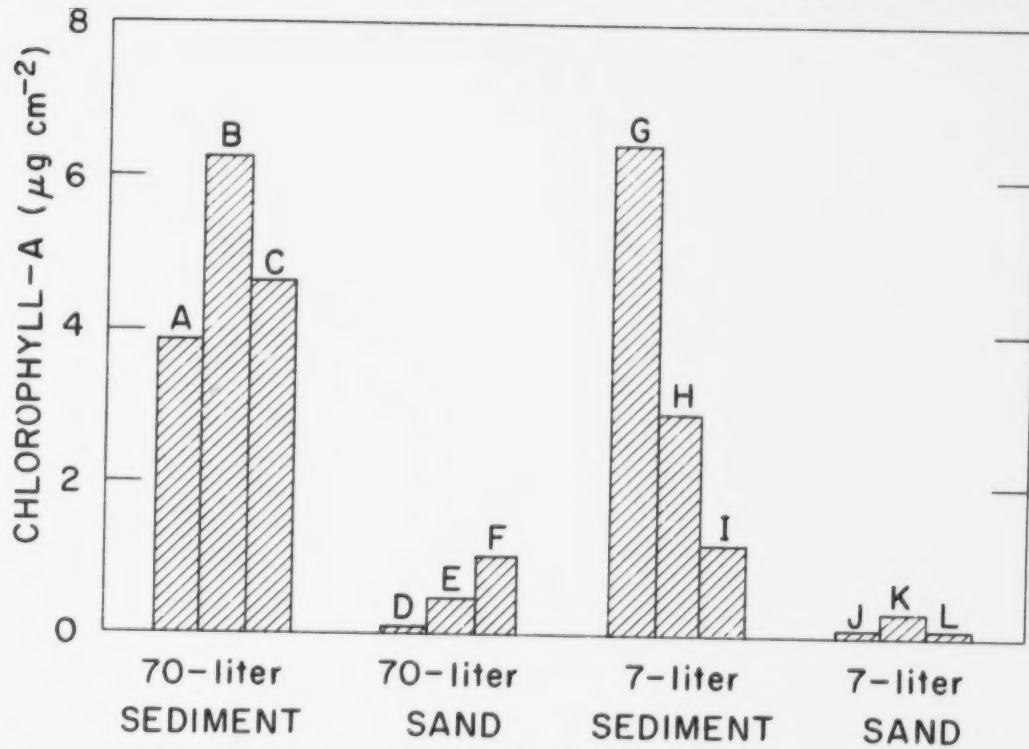


Figure 3.13. Final chlorophyll-a concentrations, Experiment 1. Bars represent replicates identified by letter.

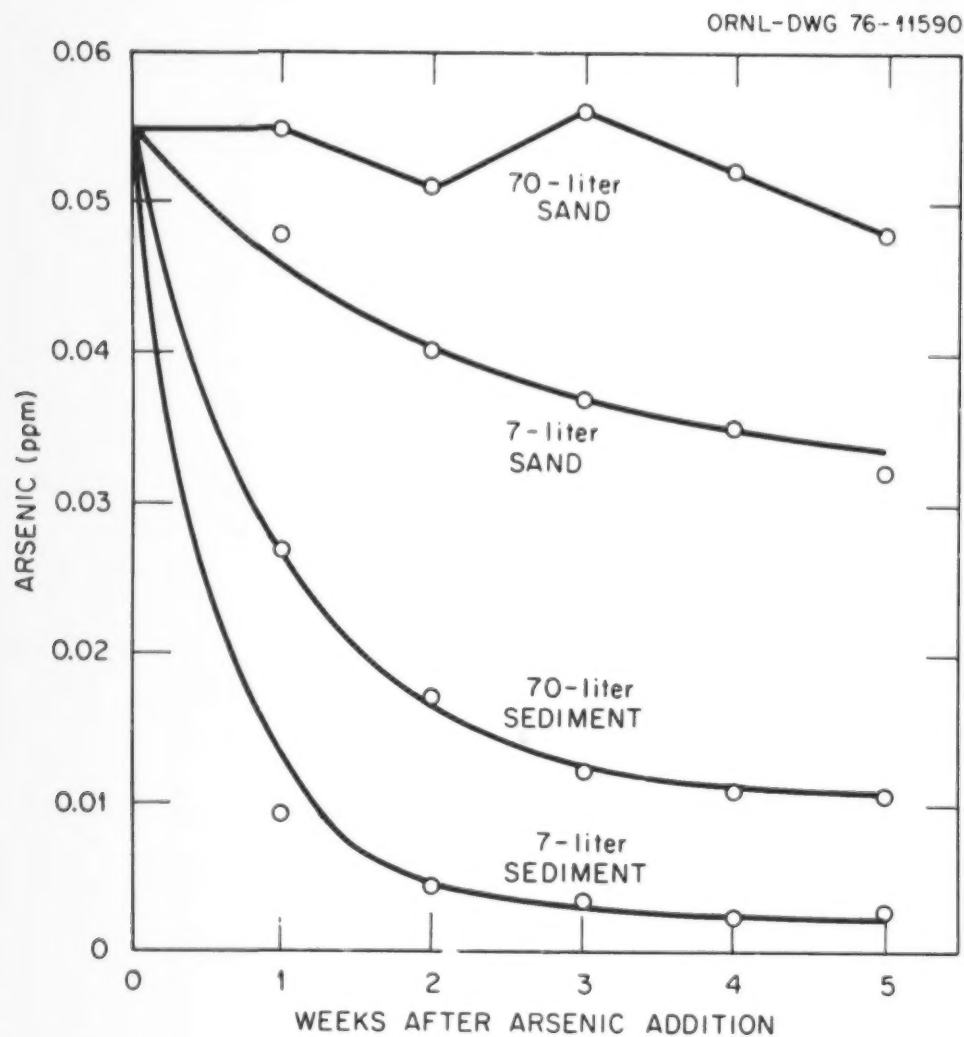


Figure 3.14. Arsenic concentrations in water, Experiment 1. Each point represents the mean of three replicate microcosms.

Table 3.8. Experiment 1 final arsenic concentrations (ppm)

Microcosm	Water	Sub- strate	Algae	B.R. ^a	Snails	B.R. ^a	Zoo- plankton	B.R. ^a
70-liter sediment								
A	0.0108	0.398	10.47	969	1.77	164	6.76	626
B	0.0125	0.507	19.09	1527	2.17	174	--	--
C	0.0070	0.683	16.17	2310	3.28	469	--	--
70-liter sand								
D	0.0495	0.174	33.81	683	0.435	8.8	8.33	168
E	0.0456	0.133	39.42	846	0.559	12.3	--	--
F	0.0481	0.180	65.74	1367	0.576	12.0	10.4	216
7-liter sediment								
G	0.0033	0.511	6.54	1982	1.53	464	--	--
H	0.0014	0.326	12.87	9193	1.44	1029	--	--
I	0.0035	0.276	6.00	1714	1.24	354	--	--
7-liter sand								
J	0.0310	0.120	11.58	374	0.366	11.8	--	--
K	0.0367	0.085	9.28	253	0.550	15.0	--	--
L	0.0293	0.106	14.26	487	0.788	26.9	--	--

^aB.R. = bioaccumulation ratio = concentration in organism/concentration in water. All concentrations are $\mu\text{g As/g dry wt}$, except water = mg As/liter .

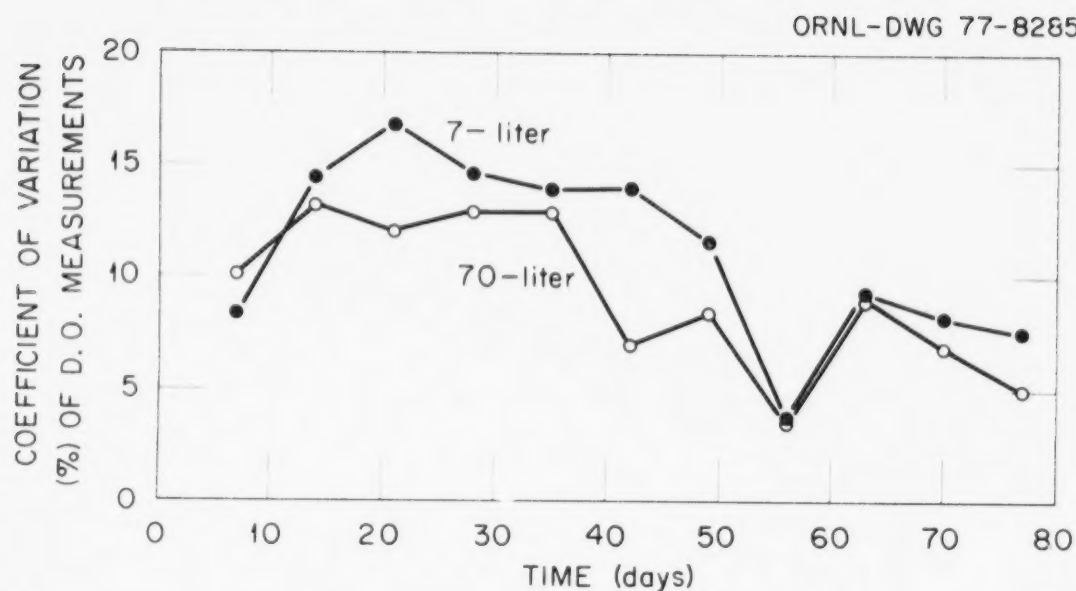


Figure 3.15. Equilibration of dissolved oxygen in 7- and 70-liter sediment microcosms, Experiment 1. Coefficients of variation among measurements made over a two-week period were calculated for each individual microcosm for 11 overlapping two-week periods. The points shown here are average C.V. from three replicate microcosms.

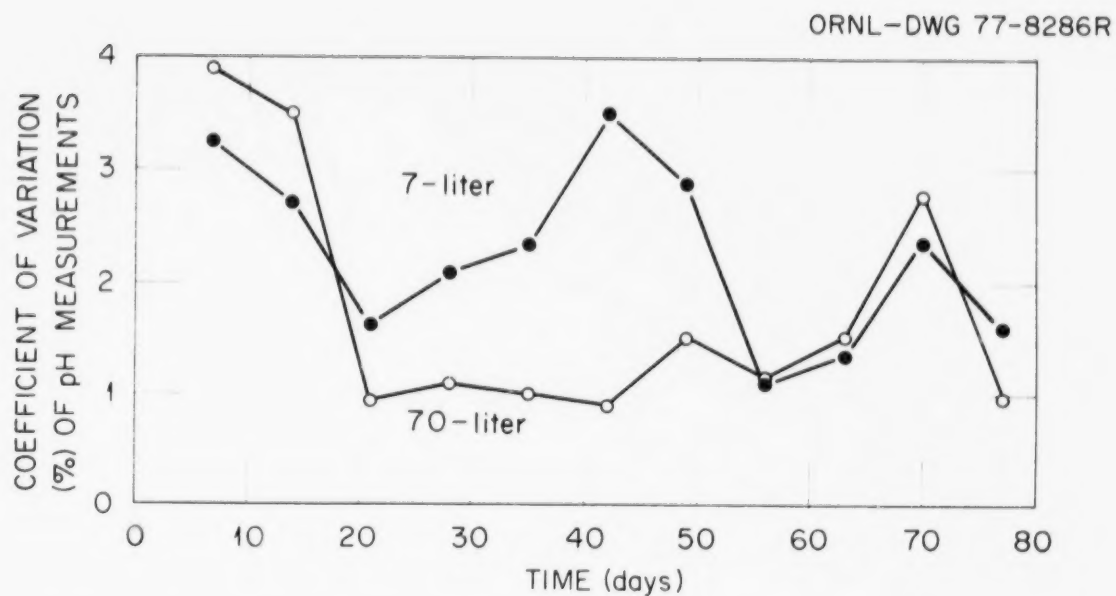


Figure 3.16. Equilibration of pH in 7- and 70-liter sediment microcosms, Experiment 1. C.V. calculated as in Fig. 3.15.

Variation between replicates was lower in the 70-liter microcosms than that in the 7-liter microcosms on the whole. Coefficients of variation among replicate 70-liter and 7-liter sediment microcosms were calculated for each of the 13 weekly water chemistry samples; the averages of these coefficients of variation are shown in Table 3.9. For total dissolved solids, alkalinity, Ca, Mg, Cl, PO_4 , and total P, 70-liter microcosms showed better replicability than 7-liter microcosms, whereas the reverse was true for K, SO_4 , NO_3 , and NH_4 . Variability of arsenic concentrations was generally greater in the small microcosms (Table 3.10).

Smaller microcosms have some obvious advantages over larger ones: more replicates can be maintained in a given space; less sediment, water, and biota are required; and the systems are movable. Small size also imposes some limitations, however, such as smaller sample sizes, and greater tendency to be disturbed by sampling and monitoring. For long-term transport studies, 70-liter microcosms are preferable, but 7-liter microcosms might be useful in experiments where repeated sampling is not necessary.

Substrate

We have used four different substrates in our microcosms: Ottawa sand (Experiment 1), sediment from local reservoirs (Experiments 1 and 4), sediment from a small fish-holding pond (Experiment 2), and sediment from spring-fed Target Range Pond (Experiments 5-9). The ecological characteristics of microcosms with sediment from the reservoirs

Table 3.9. Mean coefficients of variation (for 3 replicates) of chemical parameters in 7- and 70-liter microcosms

Variable	7-liter	70-liter	n ^a
TDS	14.73	6.18 ^b	12
Alkalinity	13.61	5.59	7
Na ⁺	5.61	7.07	12
Ca ⁺⁺	16.59	8.60	12
Mg ⁺⁺	11.73	4.90	12
Cl ⁻	21.59	10.47	12
SO ₄ ⁼	32.29	63.19	12
K ⁺	57.68	85.60	12
NH ₄ ⁺	43.08	51.34	11
NO ₃	58.01	72.62	11
PO ₄ ⁼	83.69	62.94	11
Total P	50.04	40.65	8

^aNumber of weekly C.V. averaged.

^bValues expressed as percentages.

Table 3.10. Coefficients of variation for arsenic concentrations (%) in Experiment 1

	Water	Substrate	Algae	Snails
70-liter sediment	28	27	29	33
70-liter sand	4	16	37	15
7-liter sediment	42	33	45	11
7-liter sand	12	17	21	37
Average coefficients of variation (%)				
	Abiotic components	Biotic components	Overall	
Sediment	32	29	31	
Sand	12	27	20	

and the ponds are similar (see Sections 3.2.1.1, 3.2.1.4), but microcosms with sand behaved much differently in the one experiment in which they were used. Differences in water chemistry between sand microcosms and reservoir sediment microcosms are shown in Tables 3.6 and 3.7. Microcosms with sand had far less algal growth than those containing sediment (Figs. 3.12 and 3.13), a difference reflected in dissolved oxygen (Fig. 3.10) and pH (Fig. 3.11). Apparently, sand does not supply enough nutrients to maintain closed microcosms in a stable condition; it is doubtful that the sand microcosms could have persisted for more than a few weeks beyond the termination of this experiment.

Arsenic transport in sand microcosms was affected by the low sorptive capacity of the sand (Fig. 3.14 and Table 3.8, Section 3.2.2.1). One presumed advantage of using a standard substrate material such as sand is improved replicability; Table 3.10 shows that this improvement is limited to the abiotic components of the microcosms.

Light, Temperature, and Season

We did not examine the effects of light and temperature on the microcosms. In all experiments, a bank of cool-white fluorescent tubes provided 16,000 lux on a 12-hr-light:12-hr-dark cycle. Most experiments were carried out at 16 to 18°C. Experiment 2 was at a slightly higher temperature, and in Experiment 4 (and part of Experiment 8) the environmental chamber was set at 8°C, the ambient water temperature in Target Range Pond at the time the experiment began (Table 3.11). Since temperature was only one of many variables which differed between experiments, we cannot determine the effects of temperature alone. The season

Table 3.11. Temperatures of microcosm experiments

	Temperature
Experiment 1	17-19°C
Experiment 2	19-21°C
Experiment 4	8-9°C
Experiments 5-7	16-18°C
Experiment 8	(1/25-6/9/77) 8-9°C (6/9-6/30) 12-14°C (6/30-10/6) 16-18°C
Experiment 9	16-18°C

in which a microcosm is assembled probably influences the course of ecosystem development, but the properties of mature pond microcosms converge under our usual experimental conditions (Sections 3.2.1.3, 3.2.1.4).

Macrophytes vs Algae

Although most of our microcosms were assembled with macrophyte communities, several experiments (1, 4, and 8) began with filamentous algal communities. In all microcosms containing untreated pond sediment, the mature communities were dominated by macrophytes with filamentous algae as subdominants; this occurred even in Experiment 8, where macrophytes were introduced only as propagules in the sediment. It is apparent from Figs. 3.1 and 3.6 that macrophytes utilize nutrients contained in the sediment, which is probably the reason they normally outcompete the algae. We have not performed any experiments directly comparing microcosms with macrophytes and microcosms with algae, although the data from Experiments 1 and 2 indicated that water chemistry and arsenic transport were similar regardless of the autotrophs present (Section 3.2.1.4.).

Fish

Eight 70-liter microcosms were established in July 1977 to examine the effects of including fish in the microcosm community (Experiment 5). The microcosms were constructed following our regular procedure, using water, sediment, and an Elodea community from a shallow pond. Fish were then added to the microcosms as follows:

- 2 microcosms: 10 mosquitofish (Gambusia affinis)
- 2 microcosms: 5 fathead minnows (Pimephales promelas)
- 2 microcosms: 2 bluegill (Lepomis macrochirus)
- 2 microcosms: no fish added (controls)

One of the fathead minnow microcosms became very different from the other fish microcosms and the controls, with turbid water, low pH and dissolved oxygen, low productivity, and Elodea die-offs. It was discovered in early November that a large crayfish (about 10 cm in length) had been inadvertently included in this microcosm. None of the other microcosms exhibited similar destructive effects. The other fathead minnow microcosm and the bluegill microcosms were devoid of snails, however, presumably due to predation by the fish. These three microcosms differed from the mosquitofish and control microcosms in that they had extensive blue-green algal growth on the sediment surface, dense periphyton on the aquarium walls, a thin algal mat floating on the surface, and greater growth of Elodea. It seems likely that the absence of snails (which are mainly herbivorous) rather than the presence of fish was responsible for these community effects. Zooplankton populations were dense in the control microcosms (dominated by the cladoceran Simocephalus and a cyclopoid copepod), but very sparse in the fish microcosms.

Gross primary production, as measured by the diurnal oxygen technique, averaged 7.3 ppm O_2 /day in the control microcosms, 7.9 with mosquitofish, and 12.0 with bluegill. The fathead minnow microcosm without crayfish averaged 12.1; with the crayfish, the gross primary

production was only 5.8 (Table 3.12). There were only small differences in nutrient levels among treatments, the most noticeable being elevated ammonium concentrations in all fish microcosms (except with crayfish), and somewhat more total phosphorus in the bluegill microcosms than the others (Table 3.13). The greater primary production in bluegill and fathead minnow microcosms was probably attributable to the reduced grazing pressure (due to absence of snails), but the elevated nutrient levels may also have been important.

At the end of Experiment 6, both bluegills were recovered from microcosm B1, but only one remained in B2; mean individual weights had declined by 31 to 35% over the 18-week period. Microcosms M1 and M2 contained 5 and 9 Gambusia, respectively. The mean individual weight had increased by 65% in M1 and 25% in M2. We do not have data on the fathead minnows, but live fish were still present after eight months. We conclude that fish are compatible with a stable and persistent microcosm community, although they may affect the community composition and metabolism. Snails and crayfish, on the other hand, have a significant impact on microcosm ecology.

3.2.2 Transport Studies (G. K. Eddlemon and J. M. Giddings)

3.2.2.1 Arsenic

Experiment 1

Arsenic (As) in the form of sodium arsenate, was selected as a test contaminant for our initial experiments on microcosm design. The availability of a radioisotope, arsenic-74 (half-life 17.5 days), makes

Table 3.12. Gross primary production in fish microcosms

Microcosm	n	G.P.P. ^a
C1	28	5.8 (0.1)
C2	25	8.7 (0.2)
B1	10	12.4 (1.4)
B2	11	11.6 (1.8)
M1	12	6.8 (0.7)
M2	12	9.0 (0.9)
F1	27	12.1 (1.2)
F2	25	5.8 (0.6)

^aGross primary production in ppm O₂/12 hr; mean of n measurements on equilibrated microcosms; standard deviation in parentheses.

Table 3.13. Water chemistry in fish microcosms after 12 weeks (all values are in ppm)

[illegible]

arsenic a convenient subject for studies of environmental transport. Arsenic compounds occur widely in herbicides, pesticides, industrial waste, and fossil fuels. Reduced arsenic, arsenite, has been applied to many lakes to control the growth of aquatic weeds. A recent review of the aquatic arsenic cycle indicates that "the fate of arsenic in natural waters has received little attention" (Ferguson and Gavis 1972).

Methods. Six 7-liter and six 70-liter freshwater microcosms were assembled in slate-bottomed, glass aquaria, three of each size receiving a sand substrate, and the others a reservoir-sediment substrate. The 12 microcosms were kept in an environmental chamber at 18°C. A bank of cool-white fluorescent lights provided 16,000 lux on a 12-hr-light: 12-hr-dark cycle. Appendix A provides details of microcosm construction for all experiments.

Seven weeks after the microcosms were assembled, the long-term constancy and reduced daily variation in pH, dissolved oxygen, and conductivity indicated that a condition of relative stability or equilibrium had been achieved in each microcosm, at least in terms of these parameters. At this time, a test contaminant, sodium arsenate (Na_2HAsO_4) was introduced. In this first experiment, we were primarily interested in the behavior of undisturbed microcosms and the contaminant within the undisturbed systems. Therefore, a relatively low concentration of 0.05 ppm of As was selected. The arsenate was labeled with $\text{H}_3^{74}\text{AsO}_4$ (20.4 $\mu\text{Ci/mg As}$) obtained from Amersham/Searle Corporation. A gamma spectrometer was used to count the radioarsenic activity in 2-, 5-, or 10-ml water samples removed weekly from each microcosm.

At the conclusion of the experiment, five weeks after arsenic addition, the nonbenthic filamentous algae were removed by hand from each microcosm, drained in a sieve, and weighed.

Algal subsamples were dried (55°C, 6 days) and the dry weight obtained. The dried algae were then placed into sample tubes for radioarsenic determination.

Following the removal of algae from the microcosms, two 100-ml samples from each microcosm were vacuum-filtered (Whatman GF/C) and the radioactivity of the seston on the filters was counted. Zooplankton were collected from the three microcosms containing them in sufficient numbers by straining the water through a 0.150-mm sieve. The zooplankton were washed into beakers with distilled water, filtered onto preweighed paper, dried (5 days at 55°C), weighed, and their radioactivity determined. All snails over 2 mm in length were removed from the aquaria, placed in spring water for about 1.5 hr, rinsed with distilled water, weighed, dried, reweighed, and their radioactivity counted.

Samples of the substrate from the completely drained microcosms were removed with cork borers. Three 2.21-cm² cores were placed in preweighed aluminum dishes, dried (6 days at 55°C) and reweighed. The cores were then ashed (24 hr at 550°C) to determine the organic content. Two 0.68-cm² cores from each microcosm were assayed for radioactivity.

Results. The concentrations of arsenic in the water are shown in Fig. 3.14. The decline in dissolved arsenic concentrations over the first 7 to 21 days of the experiment may be described by a simple model of logarithmic decay,

$$N_t = N_0 e^{-rt} \quad , \quad (1)$$

in which N_t = concentration at the time t , N_0 = initial concentration, and r is the first-order kinetic constant. Values of r from this and subsequent experiments are presented in Table 3.14. The rate constant for arsenic loss from water in the 70-liter sediment microcosms, 0.10/day, compared favorably with the 0.12/day calculated for similar arsenic microcosms in Experiment 2. Of the five contaminants tested under similar conditions, only mercury exhibited a higher loss rate constant (0.23/day over the first three days). Loss rate constants in 7-liter sediment microcosms averaged 0.25/day, reflecting the greater sediment surface to water volume ratio of these microcosms. In the 7-liter sand microcosms, the loss rate constants averaged only 0.018/day, due to the low affinity of sand for arsenic.

Dissolved arsenic concentrations over the entire experiment in the sediment and the 7-liter sand microcosms followed the equation

$$N_t = \frac{\lambda N_0}{\lambda + \mu} e^{-(\lambda + \mu)t} + \frac{\mu N_0}{\lambda + \mu} , \quad (2)$$

where λ = fraction lost from the water per unit time, and μ = fraction lost from the substrate per unit time (Hayes et al. 1952). The estimated values of λ and μ are shown in Table 3.15. It was not possible to fit this model to the 70-liter sand microcosms, which did not approach equilibrium before the experiment was terminated. Equation (2) was also inadequate for describing data from later experiments, leading us to adopt Equation (1) for comparative purposes.

Virtually all of the arsenic lost from the water was found in the substrate at the conclusion of the experiment (Fig. 3.17). Only 2 to 4%

Table 3.14. Summary of kinetic loss rate constants for microcosm water

Contaminant	First-order constant, r	Interval	Initial concentration, N	Remarks
Arsenic	0.10 day ⁻¹	Days 1-7	0.05 ppm	Expt. 1, 70-liter sediment microcosms
	0.12	1-24	0.066 ppm	Expt. 2, 70-liter sediment microcosms
	0.018	1-21	0.05 ppm	Expt. 1, 7-liter sand microcosms
	0.25	1-7	0.05 ppm	Expt. 1, 7-liter sediment microcosms
	0.034	1-25	9.2 ppm	Expt. 3, 500-liter pond enclosure
Selenium	0.026 day ⁻¹	Days 1-40	0.05 ppm	Expt. 6, 70-liter sediment microcosms
Chromium	0.053 day ⁻¹	Days 1-15	0.025 ppm	Expt. 4, 7-liter sediment microcosms
	0.044	1-15	0.68 ppm	Expt. 4, 7-liter sediment microcosms
	0.030	1-15	3.20 ppm	Expt. 4, 7-liter sediment microcosms
Mercury	0.227 day ⁻¹	Days 0-3	0.4 ppb	Expt. 7, 70-liter sediment microcosms
	0.091	10-41	0.1 ppb	Expt. 7, 70-liter sediment microcosms
Anthracene	0.055 day ⁻¹	Days 0-15	0.5 ppb	Expt. 8, 70-liter sediment microcosms
	0.007	20-65	0.2 ppb	Expt. 8, 70-liter sediment microcosms

Table 3.15. Adsorption and desorption parameters for arsenic in microcosms (calculated from equation 1)

	70-liter sediment	70-liter sand	7-liter sediment	7-liter sand
λ	0.83	--	1.57	0.19
μ	0.18	--	0.09	0.23

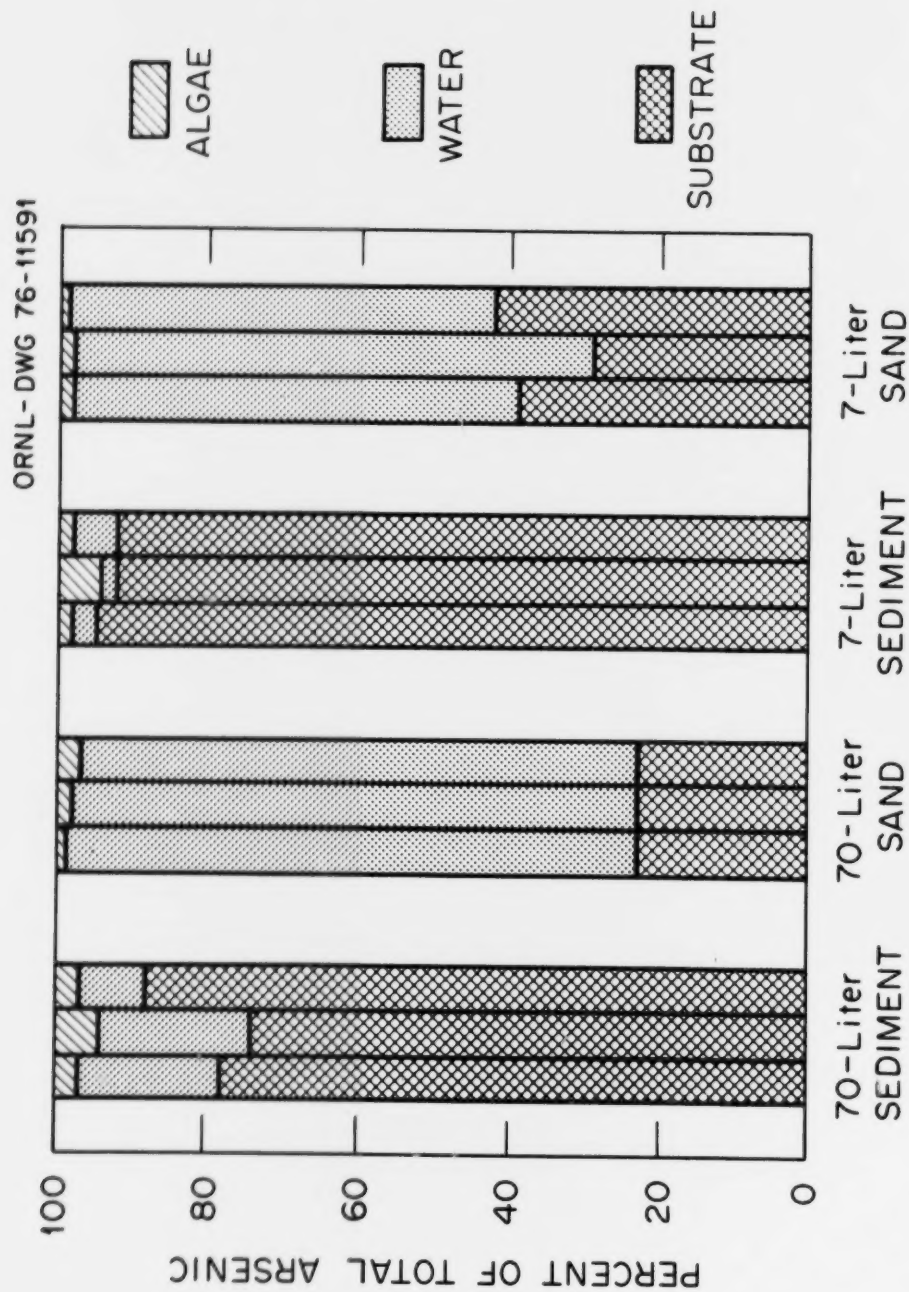


Figure 3.17. Final distributions of arsenic among microcosm components, Experiment 1.
Each bar represents one microcosm.

was found in the algae, and amounts in snails, zooplankton, and seston were negligible fractions of the total.

The final concentrations of arsenic in all microcosm components are presented in Table 3.8. Included in this table are bioaccumulation ratios (dry weight concentration in organism/concentration in water) for the biota. A lower substrate surface to water volume ratio and the relative inactivity of sand with regard to arsenic resulted in higher concentrations of arsenic in the water of the large microcosms and sand microcosms, respectively, than in the others. As a result, more arsenic was available for uptake by algae. Algal arsenic concentrations were significantly greater in the 70-liter microcosms and in the sand microcosms ($P < 0.05$) than in the 7-liter and sediment microcosms. Bioaccumulation ratios ranged from 253 to 9193 (mean 1810). Arsenic concentrations in snails were directly proportional to substrate arsenic concentration ($r = 0.947$; Fig. 3.18). If the major route of entry of arsenic into snails is via the substrate, as implied by these data, the calculated bioaccumulation ratios are meaningless.

Discussion. Table 3.16 presents published values for arsenic concentrations and bioaccumulation ratios of organisms from marine, freshwater, and laboratory habitats. These concentrations and bioaccumulation ratios are in substantial agreement with the results of the present experiment, despite the fact that the values cover a wide range of ambient arsenic concentrations and include a variety of chemical forms. Our data support the conclusion that arsenic, unlike mercury, does not accumulate in the food chain (Ferguson and Gavis 1972, Isensee et al. 1973). Within each group of organisms, the range of reported

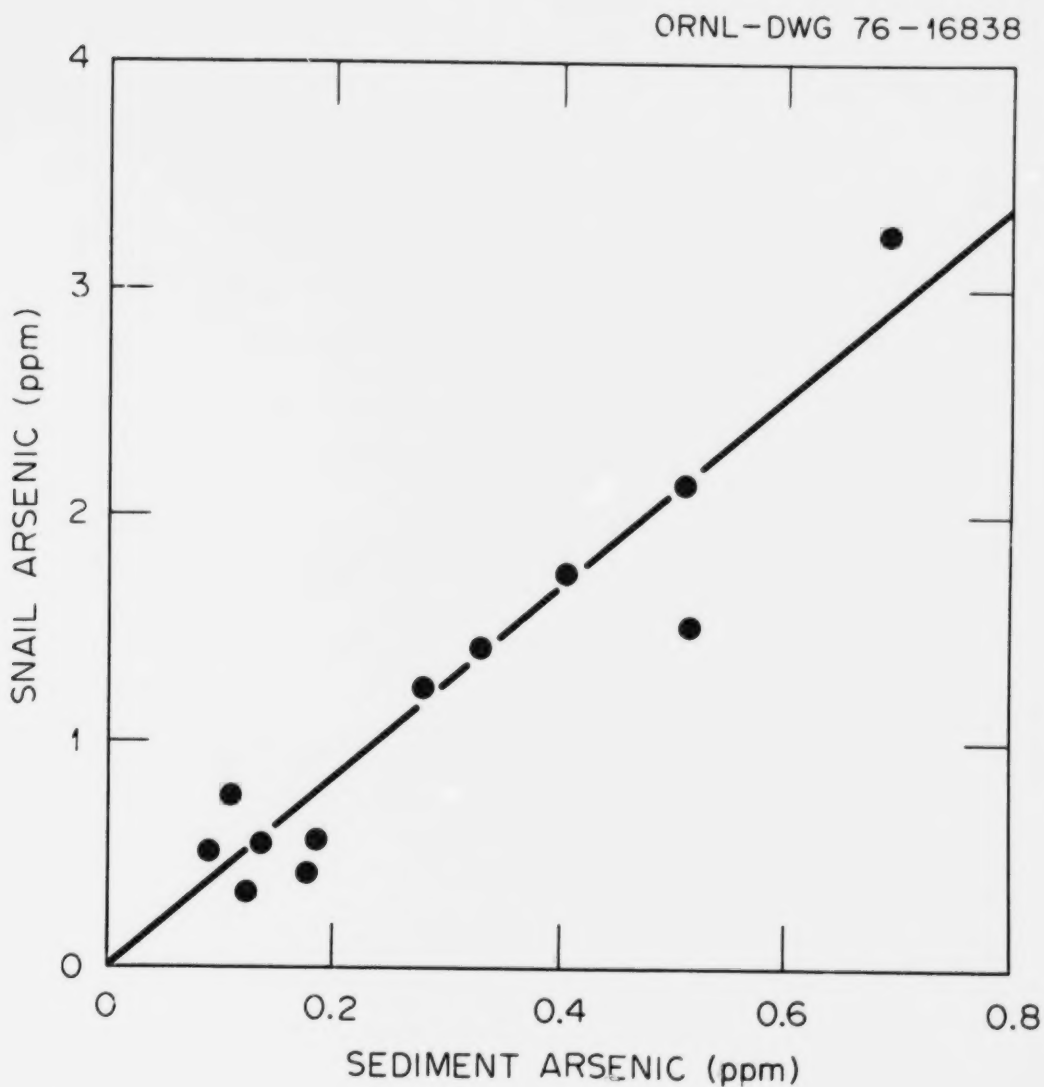


Figure 3.18. Regression of snail arsenic concentrations ($\mu\text{g As/g dry wt}$) on substrate arsenic concentrations, Experiment 1. Each point represents one microcosm. The line fitted to the data by least-squares analysis has the equation $y = 4.251x - 0.0141$ ($r = 0.9474$).

Table 3.16. Arsenic concentrations and bioaccumulation ratios from the literature

Organism	As(ppm)	Bioaccumulation ratio	Source
Plants			
<u>Oedogonium</u>	4.5-71.4	1248-1635	Isensee et al. 1973
Lake Michigan phytoplankton	5-10	1750-12,800	Seydel 1972
Lake Superior phytoplankton	3.2-4.3	---	Seydel 1972
Pond aufwuchs	11-250	6000-122,000	Sohacki 1968
Pond seston	16-250	7000-80,000	Sohacki 1968
<u>Chara globularis</u>	5-122	200-250	Sohacki 1968
<u>Myriophyllum</u>	15-63	---	Sohacki 1968
<u>Potamogeton praelongus</u>	0-15	---	Sohacki 1968
<u>Fucus</u>	12-17	3900	Penrose et al. 1975
<u>Ascophyllum nodosum</u>	10-17	3900	Penrose et al. 1975
<u>Macrocystis pyrifera</u>	72-144	---	Boothe and Knauer 1972
Filamentous algae	6-66	250-9200	This study
Molluscs			
<u>Physa</u>	1.4-2.6	299-446	Isensee et al. 1973
<u>Littorina littorea</u>	4-15	3011	Penrose et al. 1975
<u>Mytilus edulis</u>	1.6-5.4	1205	Penrose et al. 1975
<u>Physa</u>	0.3-3	8-1000	This study
Crustaceans			
<u>Daphnia</u>	5-16	736-2175	Isensee et al. 1973
Lake Michigan zooplankton	4-8	1833-4500	Seydel 1972
Lake Superior zooplankton	1.2-4.4	---	Seydel 1972
<u>Homarus americanus</u>	4-8	818	Penrose et al. 1975
Zooplankton	7-10	170-630	This study

concentrations is smaller than the range of bioaccumulation ratios; our microcosms, therefore, may be able to predict the former more accurately than the latter.

Particularly in the lake sediment microcosms, the substrate acted as the major arsenic sink. This appears to occur in nature as well. Nearly all of the arsenic entering Lake Michigan accumulates in the sediments (Seydel 1972). Of the arsenic brought to the surface from geothermal sources at Wairekei, New Zealand, only 5% remains in dissolved form; the remainder is rapidly precipitated on the mud (Ritchie 1961). Penrose et al. (1975) examined the arsenic distribution in Moreton's Harbor, Newfoundland, where arsenic-containing leachate is released from a stibnite mine. Within 1 km of the mine drainage outflow, the sediments contained 9 to 30 ppm arsenic, while the concentrations in the water were 0.2 to 2.6 ppb. Sohacki (1968) administered sodium arsenite to a small pond, and found that 60 to 70% went into the sediments in 10 days with over 90% lost after 30 days. Seydel (1972) found that the highest sediment arsenic concentrations in Lake Michigan occurred in clayey sediments (26 to 29 ppm), while the lowest occurred in sandy sediments (7.2 to 7.6 ppm). Ball and Hooper (1966) observed that sodium arsenite was taken up much faster (in aquaria) by mud substrates than by sand or gravel. In the pond studied by Sohacki (1968), areas of the bottom covered with silt adsorbed significantly more arsenic than sandy areas.

These studies all corroborate the major result of our experiment, namely, that substrate type is critical in determining the movement of arsenic into the sediments. According to Seydel (1972), the movement

of arsenic into the sediments is due to (1) coprecipitation with iron, (2) adsorption by sediment particles, and (3) adsorption by organic matter. The presence of dissolved iron in the sediment microcosms and its absence from the sand microcosms have already been mentioned. Furthermore, the lake sediment contained 5% organic matter at the end of the experiment, while the sand contained only 0.1% organic matter. These facts, together with the smaller particle size (and hence greater adsorptive surface area) of the sediment, explain the differences in substrate arsenic uptake observed (Fig. 3.17).

Experiments 2 and 3

The objectives of Experiments 2 and 3 were, in part: (1) to examine the effects of dosage level on transport and distribution of arsenic; (2) to repeat part of Experiment 1 for comparison of results (reproducibility); and (3) to compare some aspects of arsenic transport and distribution under semi-natural conditions (Experiment 3 pond enclosures) with microcosm results.

Materials and methods. Twelve microcosms were established in 70-liter glass aquaria in July 1976. Each microcosm contained 6 cm (about 10 kg) of untreated sediment from a shallow pond, 40 g (wet wt) of a mixed Elodea - Potamogeton community from the same pond, and 56 liters of spring water. A diverse assemblage of animals was present in the sediment and the macrophyte inoculum, including protozoans, rotifers, copepods, cladocerans, oligochaetes, nematodes, snails, and insects. The microcosms were maintained in an environmental chamber at 18°C on a 12-hr-light:12-hr-dark cycle. Details of the microcosm technique may be found in Appendix A.

Ten weeks after the microcosms were established, sodium arsenate was added to nine of them. Appropriate volumes of a sodium arsenate stock solution and a solution of carrier-free $\text{H}_3^{74}\text{AsO}_4$ were mixed with 100 ml of water from each microcosm. The mixture was slowly introduced into each microcosm through a glass tube so that the mixture entered in a horizontal stream approximately 10 cm below the water surface. Arsenic concentrations in the sodium arsenate stock solution and in untreated pond sediment, water, and Elodea were measured by an arc-emission technique (Feldman 1977) and used to calculate specific activities of As added at each treatment level. Arsenic concentrations in various components of the microcosms were then determined by gamma spectroscopy. The initial As concentrations in water were 0.066, 11.5, and 143 ppm, with three replicates per treatment. These concentrations bracket the range of reported toxic concentrations for most freshwater organisms (Becker and Thatcher 1973, Cushman et al. 1977). Three microcosms were left untreated as controls.

At the same time that the microcosms were inoculated with As, three of six 500-liter pond enclosures described in Appendix A, Experiment 3, also received As at an initial dose of 9.2 ppm As in water.

Water, sediment, and macrophyte samples were regularly removed from all microcosms for radioassay: water by pipette, sediments by glass tube corers, and macrophytes by forceps. Sediment and macrophyte samples were freeze-dried and weighed prior to radioassay. Final sediment samples were extracted using a cork borer of 1.7-cm^2 area after all standing water had been removed to facilitate removal of whole, intact, replicable cores. Cores were sectioned at 1.0-cm intervals to

determine arsenic distribution by depth. Less frequently we applied similar sampling techniques to the outdoor pond enclosures.

Results. As determined by an arc-emission technique (Feldman 1977), background As levels in pond sediments, water, and Elodea used in Experiment 2 averaged 4.3 ppm, 0.75 ppb, and 2 ppm, respectively. Snails (Physa), Potamogeton, and Spirogyra had less than 3 ppm. The sediment and water background levels compare favorably with the lower end of concentration ranges reported for U.S. rivers and lakes. Elodea canadensis from a New Zealand geothermal area had 3 ppm As; Potamogeton less than 6 ppm (NAS 1977). Experiment 3 pond enclosures were placed in the source pond for Experiment 2 microcosm components and therefore the same concentrations apply, except that pond water was slightly lower in As at 0.57 ppm.

Figures 3.19 and 3.20 show that dissolved As concentrations declined over time in all treatments. The 0.066-ppm (A, B, C) and 11.5-ppm (D, E, F) microcosms and the 9.2-ppm (V, W, Y) pond enclosures reached mean equilibrium concentrations of 0.007, 3.9, and 4.1 ppm, respectively, within four weeks. Arsenic concentrations in water of the 143-ppm microcosms (G, H, I) failed to equilibrate by the end of the experiment; final concentrations averaged 87 ppm.

As in Experiment 1 and subsequent experiments with selenium, mercury, and anthracene, and probably chromium, the sediments were the major As sink. Table 3.17 presents the final arsenic distribution due to added As for all Experiment 2 microcosms and Experiment 3 pond enclosures. Arsenic concentrations in the top centimeter of sediment

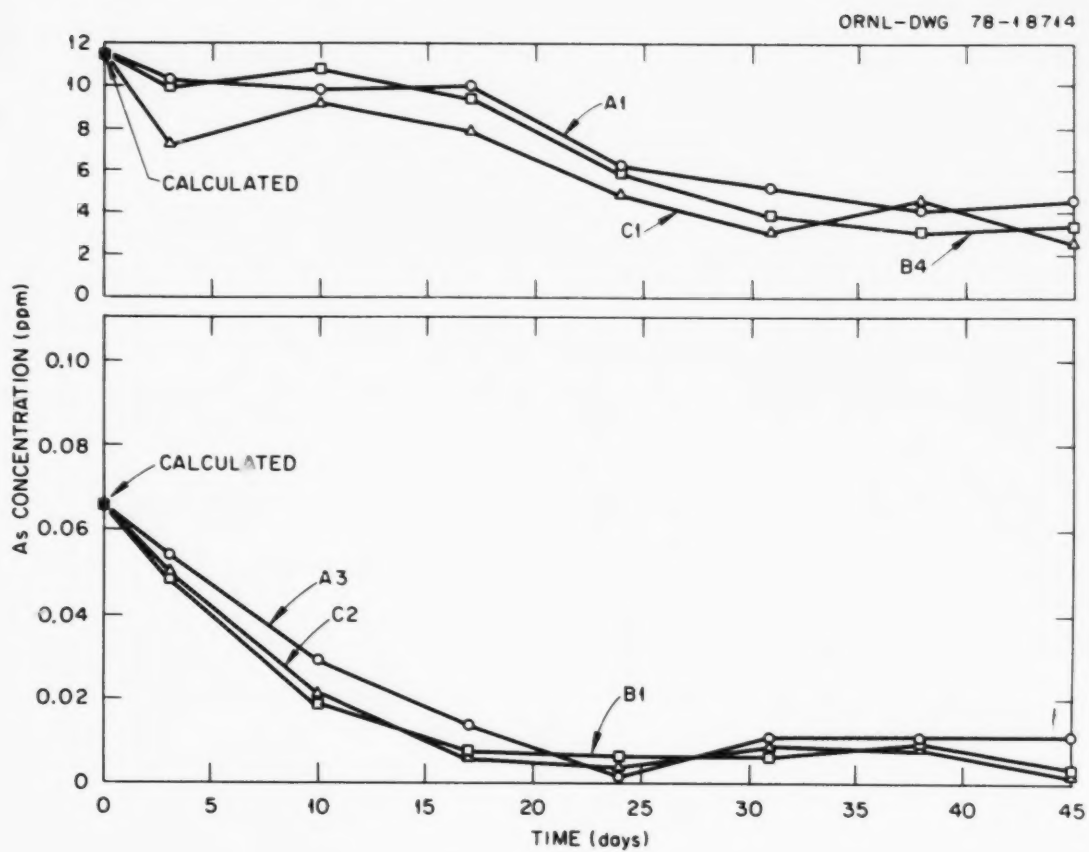


Figure 3.19. Dissolved arsenic concentrations, Experiment 2. Lower graph: 0.066-ppm treatment. Upper graph: 11.5-ppm treatment. Each point represents one sampling date for one microcosm.

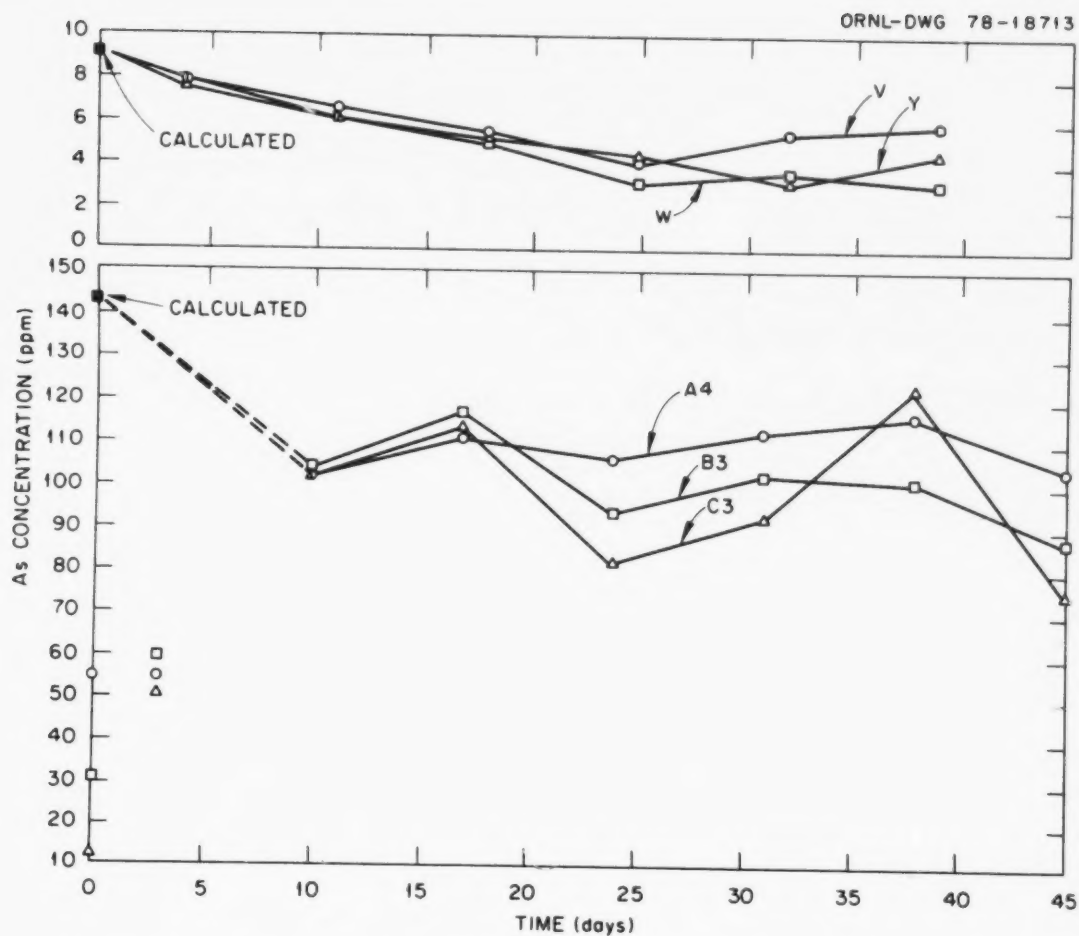


Figure 3.20. Dissolved arsenic concentrations, Experiments 2 and 3. Lower graph: 143-ppm treatment, Experiment 2. Upper graph: pond enclosures, Experiment 3. Each point represents one sampling date for one microcosm or enclosure.

Table 3.17. Final mean arsenic concentrations (ppm): Experiment 2

Microcosm (treatment)	Water ^a	Sediment (top cm)	Elodea ^b Shoots	Elodea ^b Roots	Potamogeton ^b	Phragmites ^b	Artemisia ^b	Artemisia ^b	Artemisia ^b	Artemisia ^b	Artemisia ^b
0.056 ppm											
A	0.0089	1.04	71.4 (8070)	92.0 (2430)	6.55 (740)	1.77 (330)	1.11 (100)	1.11 (100)	1.11 (100)	1.11 (100)	1.11 (100)
B	0.0064	1.22	11.5 (5120)	13.7 (2920)	1.69 (154)	1.05 (240)	1.05 (240)	1.05 (240)	1.05 (240)	1.05 (240)	1.05 (240)
C	0.0063	1.05	42.1 (7990)	13.7 (2920)	1.69 (154)	1.05 (240)	1.05 (240)	1.05 (240)	1.05 (240)	1.05 (240)	1.05 (240)
Mean	0.0063	1.10	48.7 (7960)	20.4 (2710)	4.60 (546)	1.44 (340)	1.44 (340)	1.44 (340)	1.44 (340)	1.44 (340)	1.44 (340)
11.5 ppm											
D	4.59	237	2140 (456)	—	—	—	—	—	—	—	—
E	3.47	222	2980 (656)	—	—	—	—	—	—	—	—
F	3.45	279	2910 (643)	—	—	—	—	—	—	—	—
Mean	3.87	246	2440 (652)	—	—	—	—	—	—	—	—
143 ppm											
G	103	1350	2300 (21)	—	—	—	—	—	—	—	—
H	96.4	1120	4370 (56)	—	—	—	—	—	—	—	—
I	73.5	1450	6460 (70)	—	—	—	—	—	—	—	—
Mean	97.6	1410	4380 (56)	—	—	—	—	—	—	—	—
0.2 ppm enclosures											
V	5.08	24.41	915 (160)	—	—	—	—	—	—	—	—
W	3.28	24.4	816 (750)	—	—	—	—	—	—	—	—
X	3.88	25.9	—	—	—	—	—	—	—	—	—
Mean	4.11	24.9	821 (210)	—	—	—	—	—	—	—	—

Mean equilibrium concentrations for A-F, V-X, final concentrations for G-I.

b: Dead in D-I.

Numbers in parentheses represent the bioaccumulation ratio (organism:water).

dV, W, and X sediments originally expressed as $\mu\text{g As cm}^{-2}$, converted to ppm on basis of ratios of 1 g sediment: 100 g dry weight.

averaged 1.1 ppm in the 0.066-ppm treated microcosms (A, B, and C), 246 ppm in the 11.5-ppm microcosms (D, E, and F), and 1410 ppm in the 143-ppm microcosms (G, H, I). Sediments of the 9.2-ppm treated pond enclosures yielded only 25 ppm As, about eight times lower than might have been predicted on the basis of the 11.5-ppm microcosm results. Although this large difference can be explained in part by the approximately 2.5 times greater sediment surface to water volume ratio of the microcosms, only tentative explanations can be offered for the remainder:

(1) Uptake by periphyton. Periphytic growth on the Plexiglas walls of the pond enclosures was much greater than that on the glass walls of the microcosms. Further, periphyton yielded the highest concentrations of As measured in any of the 11.5-ppm microcosm components other than surface scum.

(2) Stratification of pond enclosure water. Temperature measurements of enclosure water indicated some stratification which conceivably could have reduced contact of As with sediments.

(3) Sediment sampling errors due to difficulties in extracting replicable and representative cores.

(4) Possible leaks at enclosure seams and/or bottom.

Sediment As depth profiles are shown in Fig. 3.21. Based on core sections, the top centimeter of sediment contained 63.3% of all sediment As introduced into 0.066-ppm microcosms, 71.9% of all sediment As introduced into 11.5-ppm microcosms, and 69.2% of that introduced into 143-ppm microcosms. The top 2 cm of sediment in the first two treatments contained about 90% of all sediment As. Approximately 85% resided in the top 2 cm of sediment in the 143-ppm microcosms.

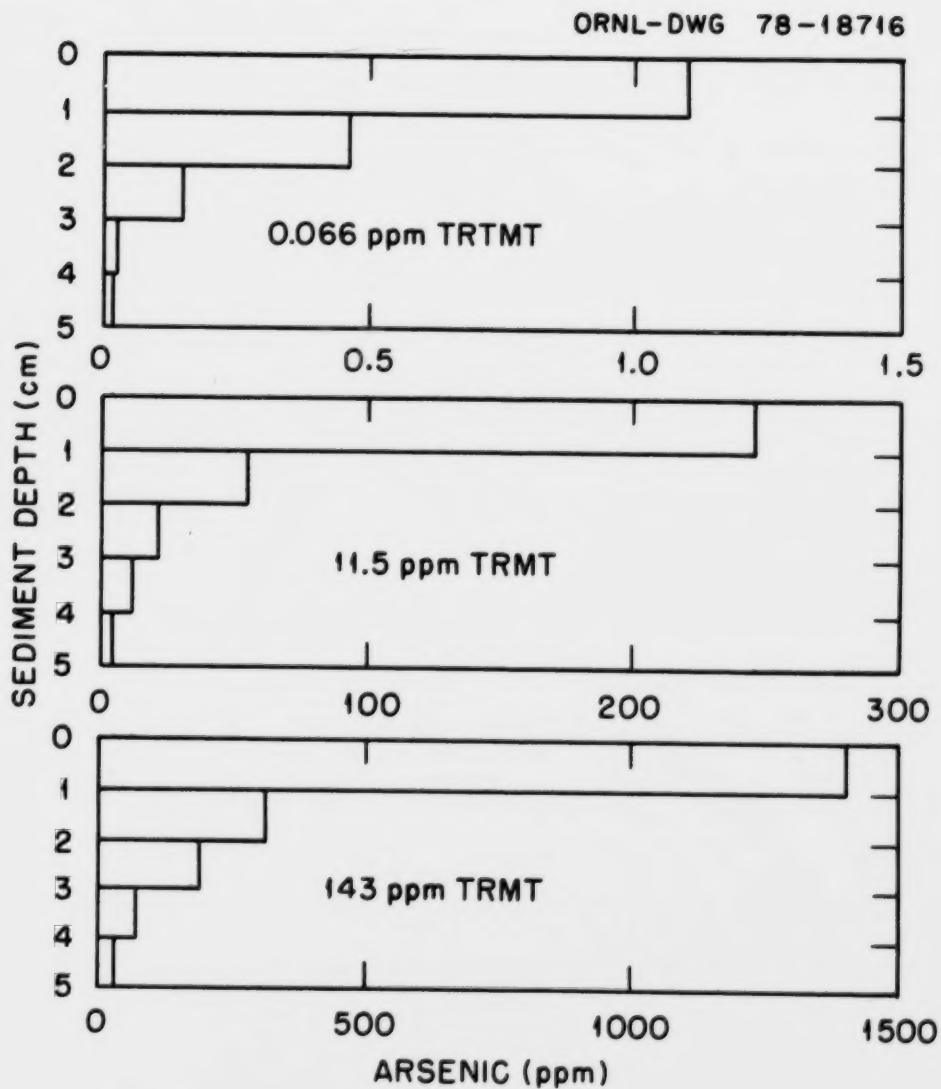


Figure 3.21. Sediment arsenic profiles, Experiment 2. Each line represents the mean of three replicate microcosms. Cores were sectioned into 1-cm intervals; points are plotted at the midpoint of each interval.

Final As mass balances are presented in Table 3.18. The distribution of As between sediment and water of 0.066-ppm microcosms is about the same as in the 0.05-ppm treated 70-liter sediment microcosms of Experiment 1 (Fig. 3.17; 80% in sediment in both cases). Although percentage values for sediment As listed for the 11.5-ppm microcosms in Table 3.18 are nearly the same as for the 0.066-ppm microcosms, the greater amounts in water as listed in the adjacent column in concert with our greater confidence in the accuracy of the water determinations, indicate that, in fact, more of the introduced As is remaining in the water at the higher treatment levels. This observation is further corroborated by the results from the 143-ppm microcosms (Table 3.18).

Elodea and Potamogeton accumulated impressive quantities of As as shown by Table 3.17. Elodea As concentrations averaged 49 ppm (bio-accumulation ratio, B.R. of 7060) in the 0.066-ppm microcosms while Potamogeton averaged 4.6 ppm (B.R. of 582). In the 11.5-ppm microcosms most macrophytes were killed. Even so, As burdens had climbed to an average of at least 2440 ppm at experiment's end, although the B.R. had dropped dramatically to 652, less than one tenth of the 7060 reported above for 0.066-ppm microcosms. The same trend continued in proceeding from 11.5-ppm to 143-ppm microcosms, except that Elodea (dead) As burdens merely doubled. Interestingly, As concentrations in Elodea from pond enclosures were only about one-third of concentrations in these plants from 11.5-ppm microcosms, even though initial enclosure water concentrations (9.2 ppm) were almost as high as in the 11.5-ppm microcosms. Algae (Spirogyra) averaged 2.1 ppm As in the 0.066-ppm

Table 3.18. Final arsenic mass balance - Experiment 2

Treatment (mg/liter)	Microcosm	% of initial As in sediment	% of initial As in water	Total
0.066	A	85.3	13.5	98.8%
0.066	B	75.8	9.7	85.5
0.066	C	80.3	8.0	88.3
	Mean	80.5	10.4	90.9
11.5	D	77.9	40.8	119
11.5	E	85.6	30.2	116
11.5	F	85.2	30.0	115
	Mean	82.9	33.7	117
143	G	39.9	71.0	111
143	H	44.2	59.6	104
143	I	45.8	50.7	96.5
	Mean	43.3	60.4	104
9.2	V	4.0	67.2	71.2
9.2	W	4.1	43.4	47.4
9.2	Y	4.8	54.1	58.9
	Mean	4.3	54.9	59.2

microcosms, considerably less than the average 15.2 ppm exhibited by algae in the 0.05-ppm microcosms of Experiment 1. The exceedingly rapid growth of algae offers one possible explanation for this difference; i.e., algae in 0.066-ppm microcosms may have bloomed late in the course of the experiment and thus had less time in which to accumulate As. This may also explain the large difference between Spirogyra As burdens in the 11.5-ppm microcosms (55-285 ppm) and those in the pond enclosures (1970 ppm).

Periphyton As burdens averaged 11 ppm in 0.066-ppm microcosms, 3500 ppm in 11.5-ppm microcosms, and 5200 ppm in 143-ppm microcosms, while the blue-green algal mats on the sediment surface averaged 4310 ppm in the 143-ppm microcosms.

Snails from 0.066-ppm microcosms averaged slightly less As (1.6 ppm) than snails (2.4 ppm) from 0.05-ppm microcosms of Experiment 1. Snails exposed to the higher concentrations of the 11.5-ppm microcosms averaged 309 ppm As. These concentrations are based on whole, dry snails including shells, and would undoubtedly be much higher if based on tissue alone.

An estimated 23 and 36 ppm As were found in mosquitofish of pond enclosures V and W, respectively, while tadpoles from enclosure Y contained a remarkable 402 ppm As. A single sample of chironomid midge larvae yielded an As concentration of 374 ppm.

The unusual shape of the early part of the 11.5-ppm microcosm concentration curves (Fig. 3.19) suggests two possible explanations:

- (1) The initial decline in As concentration due to uptake by macro- and microbiota was followed by death of much of the biota, with the

resultant release of As back to the water. Succession to a more As-tolerant community and subsequent As uptake then produced the more typical As decline observed after Day 17.

(2) The sudden decline in microcosm water pH remobilized As from sediments to which it had first adsorbed (see Section 3.2.3.2, Fig. 3.43).

The very low concentrations measured in the 143-ppm microcosms on the third day are probably a result of the heavy As solution initially sinking to the bottom of the microcosms in spite of our efforts to ensure mixing with the lighter water.

3.2.2.2 Chromium

Chromium's high potential toxicity, capacity for bioaccumulation in aquatic systems, and its presence as an important contaminant in coal conversion effluents (Hildebrand et al. 1976) justified its inclusion in our microcosm transport studies. Chromium (Cr) was the only test contaminant used in this series of studies which was not labeled with a radiotracer and which was introduced into the microcosms in a series of recurrent doses rather than in a single slug.

Methods. Eight pond microcosms of 7-liter capacity each were constructed in the same manner as the arsenic sediment microcosms. Appendix A provides further details (Experiment 4). Each microcosm received locally obtained whole river sediment (clayey; 3% organic matter) to a depth of 5 cm, approximately 4.6 liters of spring water, and a 6.0-g inoculum of wet algae, primarily Spirogyra, with the usual assemblage of snails and microorganisms. All microcosms were placed in

a growth chamber at 7 to 8°C on a 12-hr-light:12-hr-dark cycle. After a 13-week equilibration period, during which several chemical and physical parameters were monitored (Section 3.2.1), the microcosms were dosed with sufficient hexavalent chromium as Na_2CrO_4 to produce 0.1 ppm Cr in water of microcosms B1 and B2, 0.25 ppm Cr in water of microcosms C1 and C2, and 1.0 ppm Cr in water of D1 and D2. Microcosms A1 and A2 served as controls. Appropriate amounts of NaHCO_3 were added to A1, A2, B1, B2, C1, and C2 to ensure equality of sodium content among all microcosms. These treatments (same doses as above) were repeated three times at two-week intervals (Fig. 3.22). In contrast to our other microcosm experiments in which test contaminants were labeled with radioisotopes, only stable chromium was used; chromium concentrations were determined by atomic absorption spectroscopy.

Water of each microcosm was sampled regularly by pipetting three 5-ml subsamples from different locations within the microcosm, pooling, filtering through a Whatman No. 42 filter into sample vials, and acidifying with Ultrex sulfuric acid.

Sediment samples were first obtained using a glass tube. Later a No. 5 cork borer permitted greater precision in removing replicable cores after most water had been drained from the microcosms. Sediment cores were then centrifuged and the supernatant pipetted into sample vials. After oven-drying at 55°C, weighing, and grinding with mortar and pestle, sediment-chromium extractions were performed by soaking in hot analytical reagent grade HCl and HNO_3 for several hours. Extractants were filtered and stored for Cr determination via atomic absorption. Sediment chromium profiles were performed by applying the above methods to cores carefully extruded onto aluminum foil and sectioned.

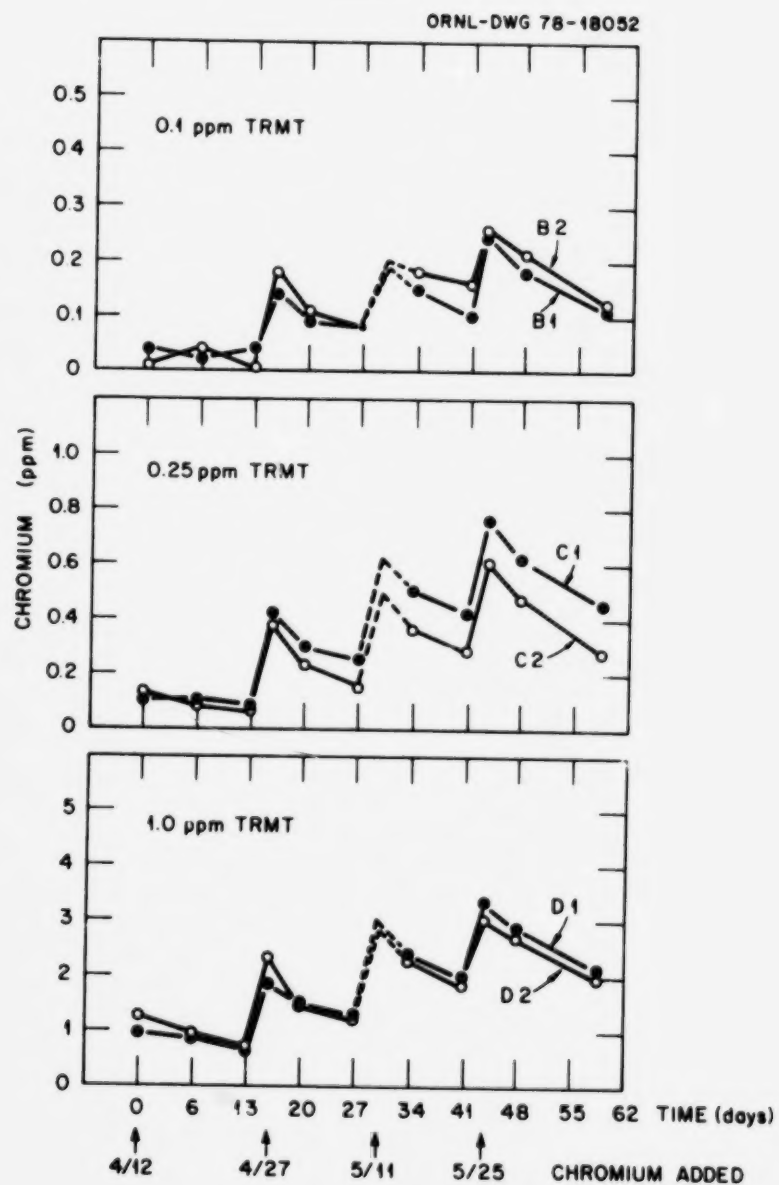


Figure 3.22. Dissolved chromium concentrations, Experiment 4. Each point represents one sampling date for one microcosm. Chromium was added at the points indicated.

Algae and snails were picked out of the microcosms with forceps, blotted dry, and ashed at 550°C. Chromium extraction proceeded as for sediments.

Results. Figure 3.22 shows, for each treated microcosm, the observed Cr concentrations in water and the points of Cr addition during the 58 days between first treatment and final sampling. After semilog plots of the concentrations (observed following the final treatment closely) approached the straight lines of logarithmic decay, Cr loss rate constants r , for water, were determined:

$$r = -\ln(n_t/n_0)/t,$$

where n_0 = initial concentration, and n_t = concentration at time t . Table 3.19 presents these loss rate constants for each microcosm. Loss rates ranged from 0.03 to 0.05/day and varied inversely with Cr concentration among the three treatments, possibly due to increased saturation of available adsorptive surfaces in the higher concentration treatments following more than 40 days of exposure. Chromium loss rates were intermediate among the five test contaminants used in this series of experiments. Final Cr concentrations in water ranged from below the limits of detection in the controls (< 0.01 ppm) to 2.1 ppm in the 1-ppm treatment microcosms, D1 and D2 (Table 3.20). Estimated chromium concentrations in rivers of the Tennessee drainage basin ranged from 2 to 20 ppb with a mean of 6 ppb (Braunstein et al. 1977). As was the case with every other contaminant tested in these studies, the sediments were probably the major sink for the introduced Cr. However, as shown by the Cr-sediment profiles in Fig. 3.23, natural Cr levels in the

Table 3.19. Chromium kinetic loss rate constants, r , for microcosm water^a

Microcosm	r
B1	0.0547
B2	0.0515
$\bar{x}$	0.0532
C1	0.0349
C2	0.0532
$\bar{x}$	0.0441
D1	0.0297
D2	0.0295
$\bar{x}$	0.0296

^aFollowing final Cr inoculation.

Table 3.20. Final Cr distribution

Treatment	Microcosm	Water (ppm)	Sediment (ppm) ^a	Algae (ppm)	B.R. ^b	Snails (ppm)	B.R. ^b
Control	A1	< 0.01	18.7(19.0)	6.2		N.D.	
	A2	< 0.01	21.0(22.5)	38.5		0.36	> 36
	$\bar{x}$	< 0.01	19.9(20.8)	22.4	2240		
0.1 ppm x 4	B1	0.11	20.7(23.0)	63.5		25.4	
	B2	0.12	22.3(19.5)	46.4		22.1	
	$\bar{x}$	0.12	21.5(21.3)	55.0	458	23.8	198
0.25 ppm x 4	C1	0.45	21.0(19.5)	179		36.1	
	C2	0.27	22.1(21.0)	290		42.2	
	$\bar{x}$	0.36	21.6(20.3)	235	653	39.2	109
1.0 ppm x 4	D1	2.14	24.5(24.0)	1020		84.7	
	D2	1.96	20.7(23.5)	1286		50.8	
	$\bar{x}$	2.05	22.6(23.8)	1150	560	67.8	33

^aBased on sum of sections; whole core values in parentheses.

^bNonequilibrium bioconcentration ratio.

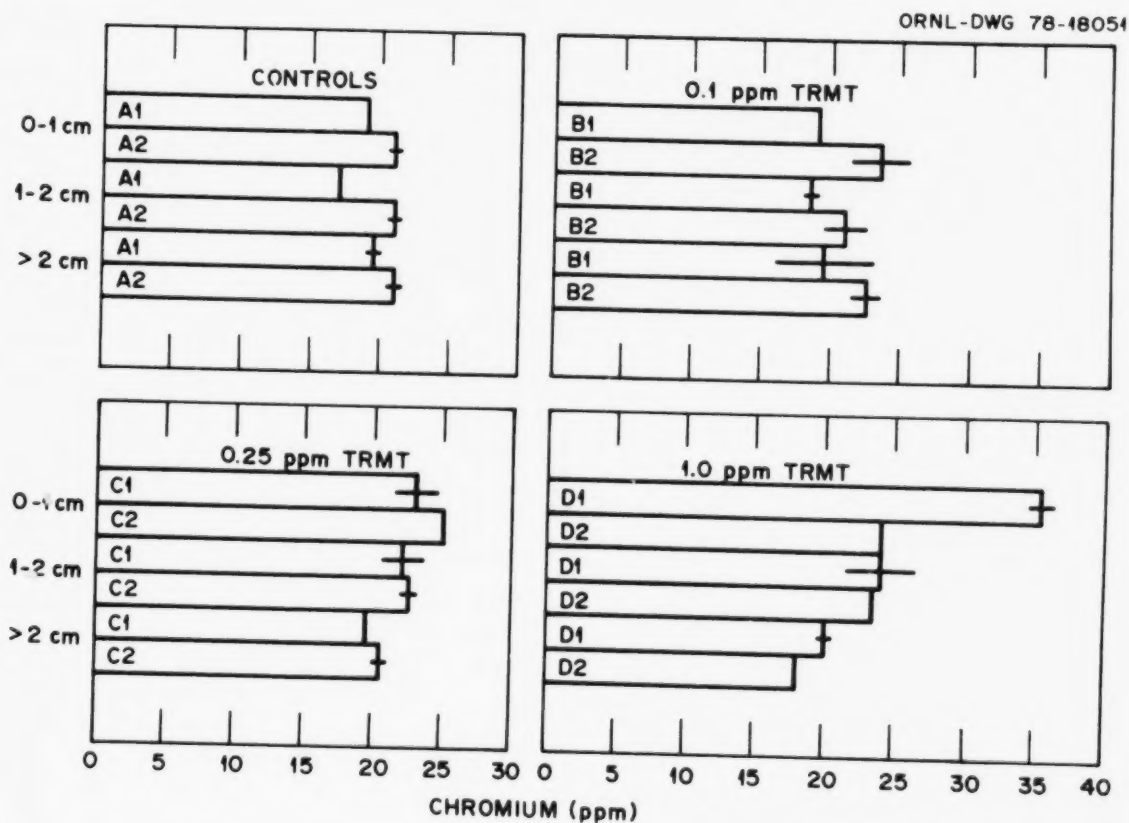


Figure 3.23. Sediment chromium profiles, Experiment 4. Each bar represents one depth interval for one microcosm.

sediments were far greater (~ 20 ppm) than the increase due to introduced Cr and, in concert with the considerable variability among sediment samples, thus tended to mask this increase. Use of a radiotracer such as ^{51}Cr would have avoided this problem. In any event, a crude mass balance based on the mean Cr concentrations in sediments of controls and the 1-ppm treatment microcosms (D1 and D2) was attempted. At the end of the experiment, an estimated 51% of Cr introduced into the water remained there, while 29% found its way into sediments, leaving nearly 20% unaccounted for.

Algae accumulated considerable quantities of Cr naturally present in the control microcosms (Table 3.20) even though dissolved Cr was less than 0.01 ppm. Probable errors associated with chemical analysis of the very small quantity of algae obtained from control microcosm A2 may explain the wide difference between Cr estimates in A1 algae (6 ppm) and A2 algae (38 ppm). All algae compare favorably with a reported value of 3.5 ppm for phytoplankton (NAS 1974). Algal Cr burdens in the treated microcosms were roughly proportional to the dose administered (Table 3.20). Algae in the two microcosms receiving the most Cr, D1 and D2, attained concentrations of 1020 and 1286 ppm, respectively. Such high concentrations without observable injury are not unknown among some of the lower terrestrial plants (NAS 1974).

Chromium concentrations in whole, dry snails (Physa) varied with level of Cr treatment but not at all linearly, as revealed by both absolute concentrations and concentration factors presented in Table 3.20. As snail Cr concentrations increased with increasing treatment levels, bioconcentration factors, $[\text{snails}]/[\text{water}]$, decreased.

Although the microcosms yielded some useful information regarding the gross behavior and distribution of Cr in semi-natural "pond" conditions, the extent and replicability of data suffered from the adverse effects of small size (7 liter) on sampling and system stability.

3.2.2.3 Selenium

We chose selenium as a "test" trace element for a transport study because of its toxicity, apparently high bioaccumulation potential, and occurrence in coal, coal ash, and coal conversion effluents (Hildebrand et al. 1976, Bolton et al. 1975). Gissel-Nielsen and Bisbjerg (1970) reported that selenate in particular is the chemical species most available to agricultural plants. Its stability, solubility, and high biological activity (NAS 1976) further underscore the need to study its transport and fate in aquatic systems. Specifically, we chose to follow for seven weeks the gross transport of selenium in lentic freshwater microcosms after having been introduced in a single "slug" as Na_2SeO_4 .

Materials and Methods

The microcosms used in this study were constructed in July 1977. Each of four 70-liter aquaria received a 5-cm layer of sediment, 60 liters of pond water, and 100 g (wet wt) of Elodea from a spring-fed pond on the Oak Ridge Reservation. The sediment and Elodea provided an inoculum of the fauna and flora in the pond, including protozoa, rotifers, copepods, cladocerans, nematodes, oligochaetes, algae, insect larvae, and, in one case, a salamander. In addition, two of the microcosms, M1 and M2, received ten mosquitofish (Gambusia affinis) each. Microcosms B1 and B2 received two bluegill (Lepomis microchirus) each. The fish fed only on food produced by each microcosm.

The microcosms were maintained in an environmental chamber at 17°C on a 12-hr-light:12-hr-dark cycle and monitored regularly for dissolved oxygen, pH, specific conductance, and other chemical parameters. The microcosms were allowed to stabilize for six weeks before introduction of selenium. Into each microcosm 3.09 mg Se as Na_2SeO_4 radiolabeled with 30 μCi of ^{75}Se (specific activity = 2.16×10^7 dpm/mg Se) were introduced via a funnel apparatus which facilitated even and gentle distribution throughout the water column. These concentrations provided initial conditions of 1100 dpm/ml and 0.0515 ppm selenium in water.

The sampling regime was a compromise between one which would allow frequent selenium assay of all major components and one which would keep disturbances in the microcosms to a minimum. Thus, water and Elodea tips could be removed regularly, sediments and Elodea roots less frequently. Snails were captured alive, weighed, counted, and returned to their respective aquaria. Fish were not removed until the end of the experiment. Plant samples were obtained with long-handled tongs and forceps. These were divided into tips (defined as last 2.5 cm), stems, and roots. All samples were washed with distilled water, dried for 24 hr at 60°C, weighed, and transferred to counting tubes. Sediment cores were collected from the top 1.0 cm of the sediment surface, and were also dried, weighed, and placed in counting tubes. Water was filtered through 0.45- μ Millipore filters, and 1.0 ml was placed into counting tubes.

All samples were radioassayed via gamma spectroscopy using a well-type NaI crystal. The counting data were corrected for isotope decay and graphed as dpm/g over time.

At the termination of the experiment, the microcosms were taken apart, and additional components (fish, dragonfly nymph, snail eggs) were counted, freeze-dried, and weighed. Snails were dissected into flesh and shell components. Final selenium concentrations in all components were computed and mass balance and distribution tables prepared.

Results

Comparison of the kinetic rate constants of the different test contaminants in Table 3.14 reveals that selenium (0.026/day) moved out of the water the slowest. Seven weeks after introduction of selenium into microcosm waters, the concentration dropped from the initial 0.0515 ppm to 0.009 to 0.0165 ppm (Table 3.21), an 82 to 68% loss. Further, the concentration curves for both water (Fig. 3.24) and sediment (Fig. 3.25) indicate that dissolved selenium would have continued to move from water to sediment had the experiment been allowed to continue. Based on filtration of some water samples, suspended particulates generally accounted for less than 5% of selenium in whole water.

Not surprisingly, the sediments proved to be the major sink for selenium. After seven weeks, the estimated fractions of the total, introduced selenium present in the sediments ranged from 57 to 89% with a mean of 76% (Table 3.22). Final selenium concentration estimates in sediments ranged from 3.9 to 4.9 ppm dry wt. Variability among sediment samples within and between microcosms was high due to sediment heterogeneity and difficulties in sampling these wet, rather oozy, sediments.

The macrophyte community in each microcosm was composed almost exclusively of Elodea. Figures 3.26, 3.27, and 3.28 present Se

Table 3.21. Final Se concentrations ($\mu\text{g/g}$ dry wt)

	Microcosm			
	B1	B2	M1	M2
Component				
Sediment	4.92	4.87	4.41	3.86
Water	0.0090	0.0101	0.0133	0.0165
<u>Elodea</u>				
Tips	3.13	3.79	2.67	4.78
Stems	3.39	3.94	2.30	4.44
Roots	3.87	4.42	3.61	5.53
Algae	11.5	9.13		
Snails				
Whole	--	--	4.34	--
Flesh	--	--	9.66 (wet)	--
Fish	3.23	2.81	3.52	6.37
Fish gut	13.0	11.5	--	--
Dragon fly	--	5.74	--	--

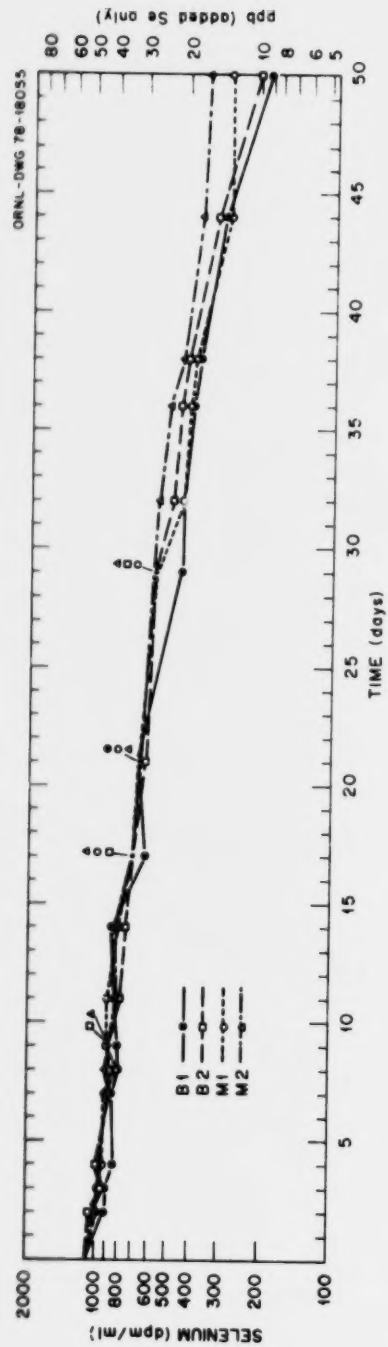


Figure 3.24. Dissolved ^{75}Se concentrations, Experiment 6. B1 and B2 contain bluegill, M1 and M2 contain mosquitofish.

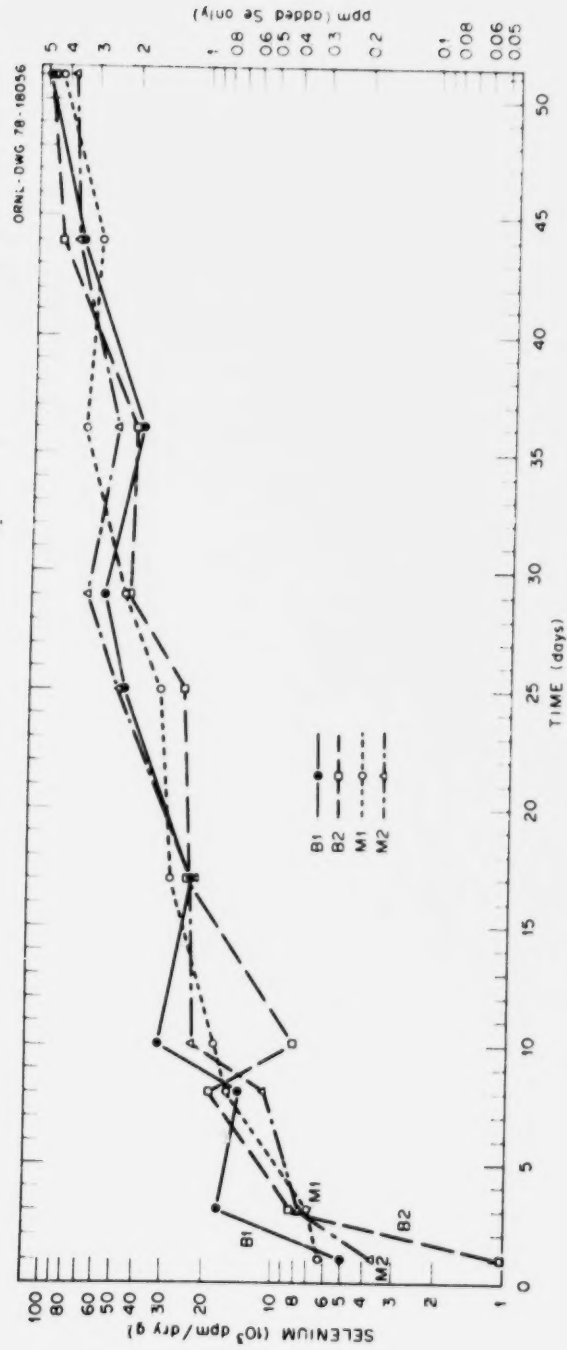


Figure 3.25. Sediment ^{75}Se concentrations, Experiment 6. B1 and B2 contain bluegill, M1 and M2 contain mosquitofish.

Table 3.22. Final Se mass balance

Component	Microcosm				Mean for all microcosms (%)
	B1 (%)	B2 (%)	M1 (%)	M2 (%)	
Sediment	89.4	85.7	70.1	57.0	75.6
Water	17.6	19.8	26.1	32.4	24.0
<u>Elodea</u>	2.97	2.62	2.07	3.89	2.89
Algae	0.17	0.17	--	--	0.08
Snails	--	--	0.30	0.30 ^a	0.30
Fish	<u>0.14</u>	<u>0.07</u>	<u>0.008</u>	<u>0.027</u>	<u>0.06</u>
Total	110.3	108.4	98.6	93.6	102.9

^aAssumed same value as M1.

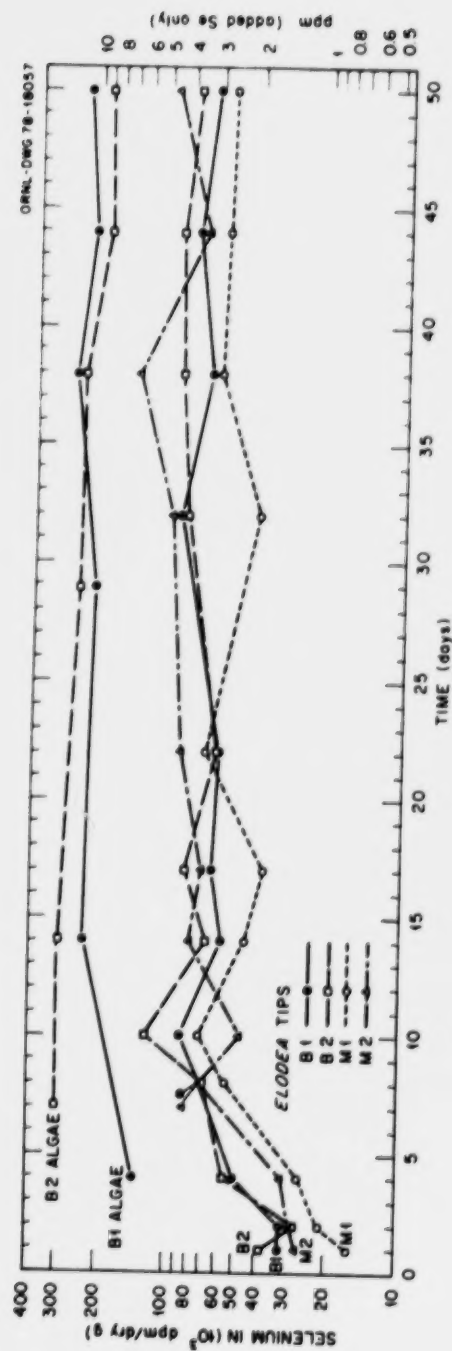


Figure 3.26. ^{75}Se concentrations in algae and Elodea tips, Experiment 6.

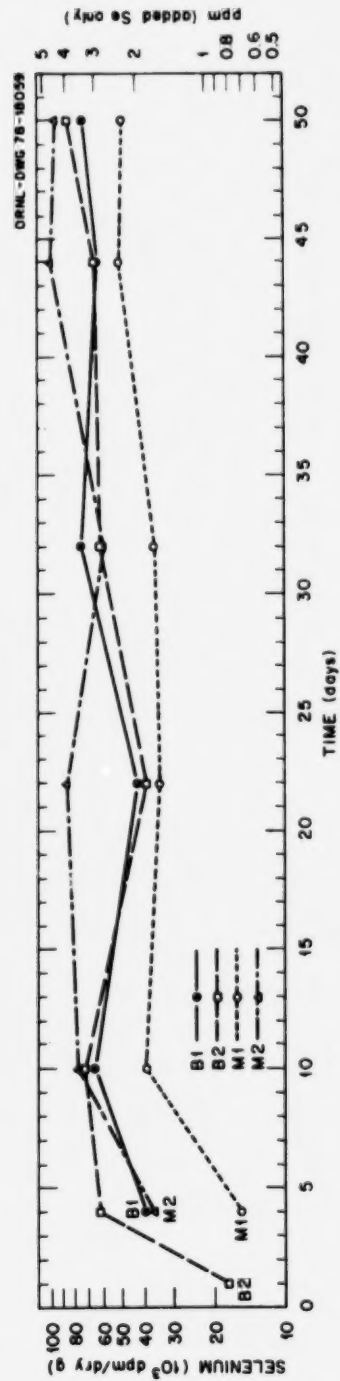


Figure 3.27. ^{75}Se concentrations in Elodea stems, Experiment 6.

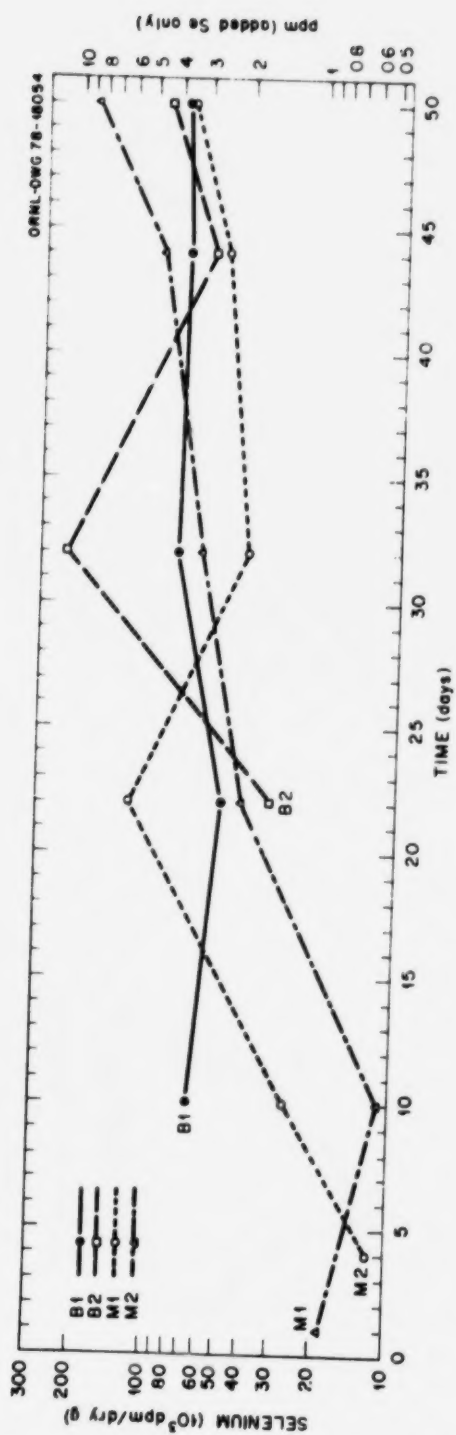


Figure 3.28. ^{75}Se concentrations in *Elodea* roots, Experiment 6.

concentrations over time for Elodea tips, stems, and roots. These plants provided the only significant sink for selenium besides sediment, accounting for about 3% of the total selenium added to all microcosms (Table 3.22). Final selenium concentrations in Elodea tips (terminal 25 mm of shoot), stems, and roots ranged from 2.3 to 5.5 ppm dry wt and are presented in Table 3.21 for each microcosm. Roots had slightly higher final concentrations of selenium than the remainder of the plants in all microcosms, possibly due to sediment contamination rather than a greater uptake of selenium. With the possible exception of roots, uptake in these plants appeared to have equilibrated with elimination and water loss rates of selenium (Figs. 3.26, 3.27, 3.28).

Bluegill predation on snails (Physa) in microcosms B1 and B2 apparently removed all grazing pressure on algae (primarily Spirogyra) since algae occurred in measurable quantities in these two microcosms alone. Selenium uptake by algae had apparently equilibrated with selenium loss rates from water by the tenth day following introduction of the contaminant (Fig. 3.26). The concentrations were considerably higher in algae than in any other component (11.5 ppm dry wt in B1, and 9.13 ppm dry wt in B2).

Snails were limited to microcosms M1 and M2, apparently due to predation by bluegills in B1 and B2. Uptake of selenium (Fig. 3.29) appeared to have equilibrated with elimination and loss from water after 27 days exposure. Final concentrations (dry wt) were estimated to be 4.3 ppm for snails of M1 and 3.4 ppm for snails of M2. The flesh of several M1 snails was removed, pooled, and radioassayed, with the result that nearly 10 $\mu\text{g/g}$ (wet wt) was selenium. On the assumption that snail

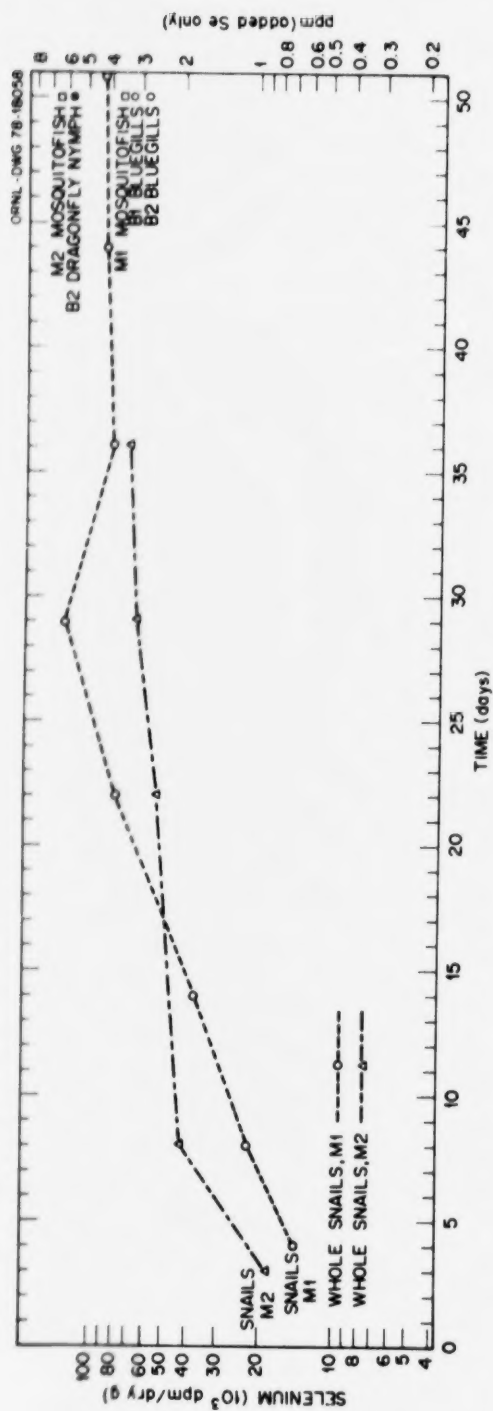


Figure 3.29. ^{75}Se concentrations in fish and invertebrates, Experiment 6.

flesh dry wt/wet wt ratio is comparable to that of fish (0.21), this would yield a remarkable 46 ppm Se in dry flesh.

Due to difficulties in sampling fish without seriously disturbing the microcosms, only final selenium concentrations were determined for these animals. Selenium burdens in fish were comparable to those in Elodea and snails, ranging from 2.8 to 6.4 $\mu\text{g/g}$ dry wt. The final distribution of selenium between water and sediment in the four microcosms as shown in Table 3.22 suggests that bluegills and/or mosquitofish may have influenced this distribution. Possibly the sediment disturbing activities of the much larger bluegill accelerated adsorption of dissolved selenium onto temporarily suspended sediment particles.

3.2.2.4 Mercury

We selected mercury (Hg) as our fourth test contaminant in this series of transport studies because of its well-documented mobility, toxicity, propensity to be concentrated by many organisms, and the manifold routes of entry into aquatic environments existing today, such as through agricultural and industrial applications and the burning or conversion of fossil fuels.

Materials and Methods. The microcosms were constructed in July 1977 from water, sediment, and organisms collected from a spring-fed pond on the ORNL reservation. The same set-up procedures and conditions discussed in detail in the previous section on selenium transport were followed with the exception that fish were excluded from the 70-liter microcosms. Further details are given in Appendix A. Five months after the microcosms were constructed (December 12, 1977), a solution of

$\text{Hg}(\text{NO}_3)_2$ radiolabeled with ^{203}Hg (specific activity = 2.84×10^6 decays/min/ μg Hg) was introduced into each microcosm sufficient to establish an initial concentration of 1.2×10^6 dpm/liter (^{203}Hg) and 0.42 μg /liter (ppb) as stable Hg.

Water, sediment, macrophytes (Elodea), snails (Physa), and zooplankton were regularly sampled and assayed for Hg, using the same methods described in previous experiments. Upon termination of the experiment, algae and water mites were also collected and assayed.

Results. Mercury moved out of the water faster than any other contaminant tested. Plots of ^{203}Hg concentration in water vs time (Fig. 3.30) for the two microcosms were fitted into two components each: one for the first three days of Hg loss and one for Days 10 to 41. First-order rate constants of 0.260 and 0.194/day were determined for the first components of microcosms C1 and C2 respectively. Loss rates declined to 0.094 and 0.089/day for C1 and C2, respectively, for the remainder of the experiments.

As expected, ^{203}Hg concentrations in the sediment rose concurrently with decreasing concentrations in water (Figs. 3.30 and 3.31), although apparently not as smoothly, as a result of the difficulty in removing whole cores of sediment intact and due to the heterogeneous nature of the sediment itself. Up to 31% of the Hg introduced into the system could not be accounted for (Table 3.23). Figure 3.31 indicates that sediment Hg concentrations had attained a rough equilibrium after seven days in microcosm C1 and after 16 days in C2. At 8.6 ppb in the top centimeter of C1 and 8.1 ppb in C2 (Table 3.24), final sediment Hg

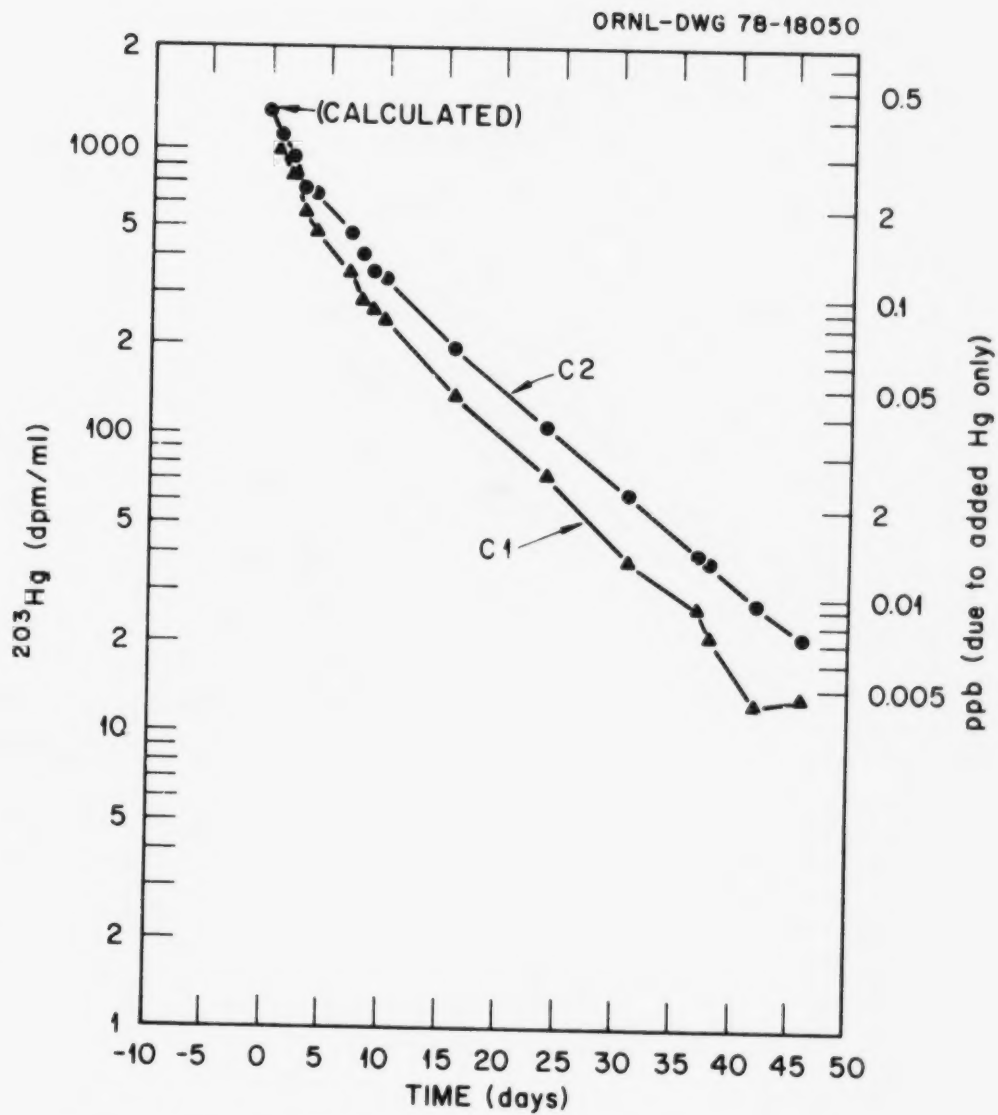


Figure 3.30. Dissolved ^{203}Hg concentrations, Experiment 7. C1 and C2 are the two replicates.

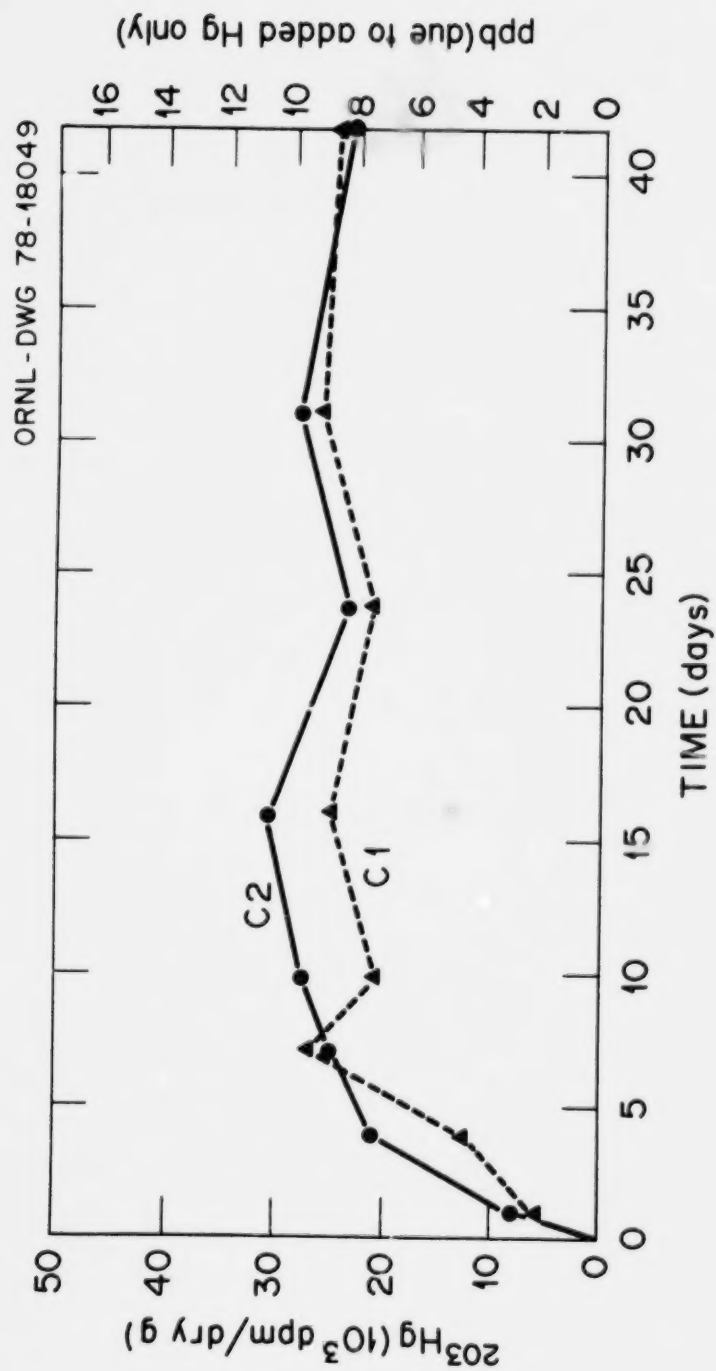


Figure 3.31. Sediment ^{203}Hg concentrations, Experiment 7, top cm only. C1 and C2 are the two replicates.

Table 3.23. Final Hg mass balance

	C1		C2	
	% of total Hg added	% of total Hg remaining	% of total Hg added	% of total Hg remaining
Water	1.1	1.5	1.7	2.5
Sediment	68.3	90.9	60.6	88.5
<u>Elodea</u> ^a	5.3	7.1	5.7	8.3
Snails	0.40	0.5	0.53	0.8
Total	75.1	100.0	68.5	100.1
Missing	24.9		31.5	

^aAssuming 23 dry g Elodea/microcosm as observed in Experiment 6 (Se).

Table 3.24. Final Hg concentrations in microcosm components due to initial dose of 0.42 ng/ml (ppb) in water

Microcosm component	C1 ng/g (ppb)	C2 ng/g (ppb)
Water	0.0046	0.0074
Sediment		
Top	8.6	8.1
Mid	3.2	3.3
Bottom	2.6	2.5
<u>Elodea</u>		
Tips	64	73
Stems	42	41
Roots	40	51
Algae	86	--
Snails		
Whole	48	43
Meats	540	370
Shell	6.3	6.3
Eggs	15	--
Zooplankton	240 (wet)	--
Water mites	130	--

concentrations (due to introduced Hg only) were slightly lower than peak concentrations measured 25 to 30 days earlier. Final average concentrations for mid-depth and bottom sediments are also presented in Table 3.24.

As shown in Figs. 3.32 and 3.33, Eloдея rapidly accumulated Hg, with peak concentrations in the actively growing tips (224 ppb in C1 and 229 ppb in C2) occurring after three days in both microcosms. The subsequent decline in Hg concentrations in both tips and stems after three or four days reflected Hg losses from water (Fig. 3.30). After an initial period of wide fluctuations in our estimates of mercury concentrations in Eloдея roots (due in part to the difficulty in sampling roots), Hg concentrations as expected did not decline, but rather remained fairly level (C1) or slowly increased (C2) as sediment concentrations increased. Bioconcentration ratios over time are presented in Fig. 3.34.

Snail tissue accumulated Hg nearly as rapidly as macrophytes (Fig. 3.35). Maximum mean Hg concentrations in snails were attained in microcosm C1 after nine days exposure (933 ppb dry wt). Highest observed concentrations in C2 snail tissues were observed after four days (484 ppb). In contrast to what was observed in macrophyte tips, concentrations remained fairly level after 16 days until the end of the experiment. This suggests that, whereas the Eloдея tips obtained most of their Hg burden from the continually declining Hg in water, the snails were accumulating Hg primarily from food in and on the sediment or from the sediment itself. Final mean Hg concentrations in dry snail tissue after 30 days exposure were 545 ppb in C1 and 365 ppb in C2. Snail Hg bioconcentration curves are plotted in Fig. 3.36.

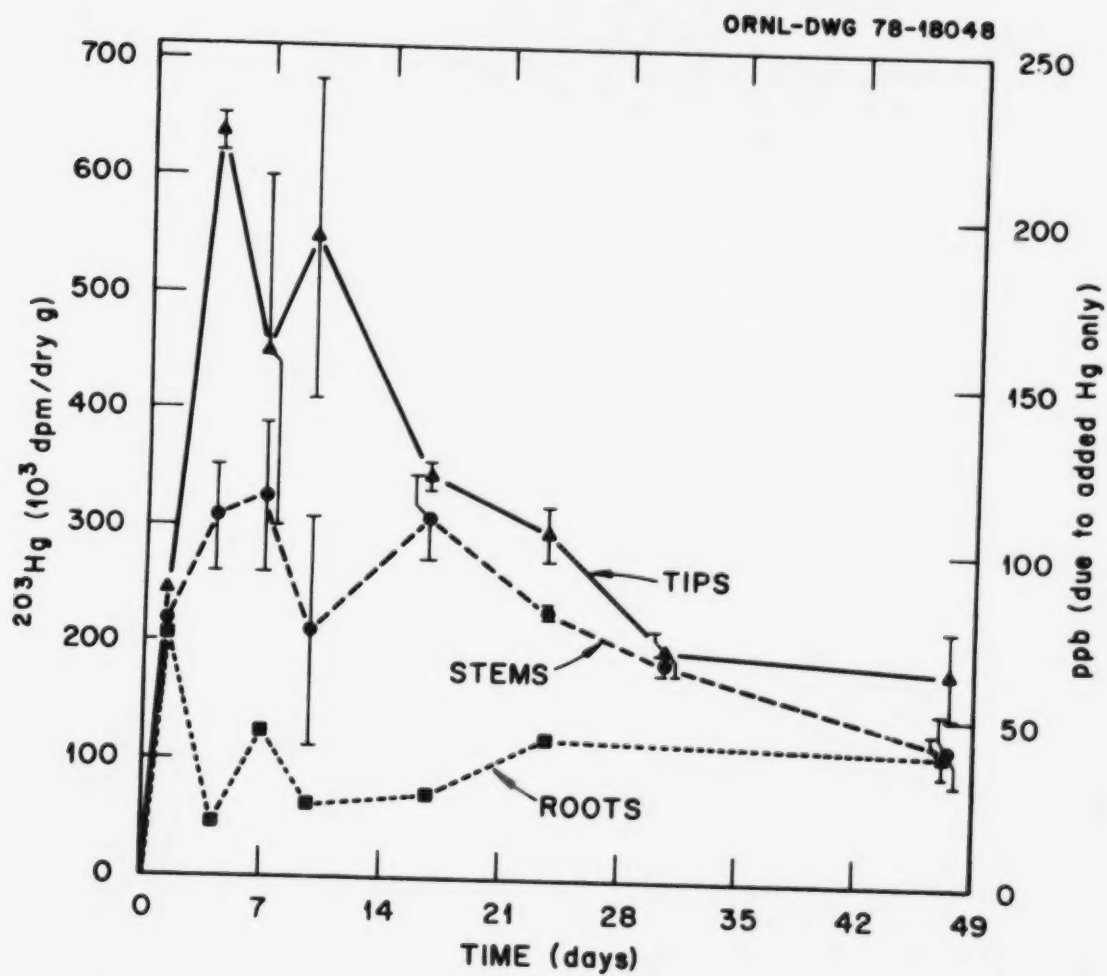


Figure 3.32. Elodea ^{203}Hg concentrations, Experiment 7, replicate C1.
 Bars around each point indicate 1 S.D.

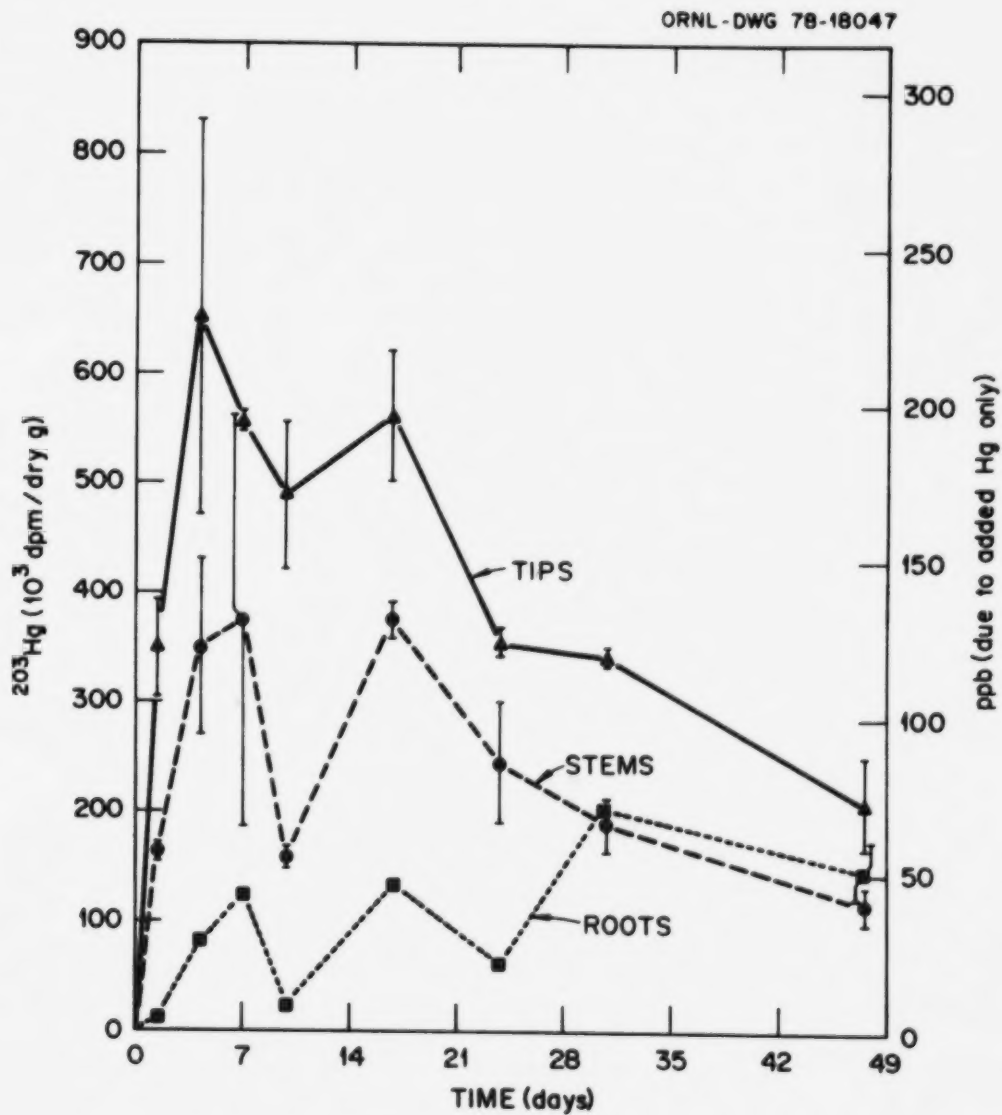


Figure 3.33. Elodea ²⁰³Hg concentrations, Experiment 7, replicate C2. Bars around each point indicate 1 S.D.

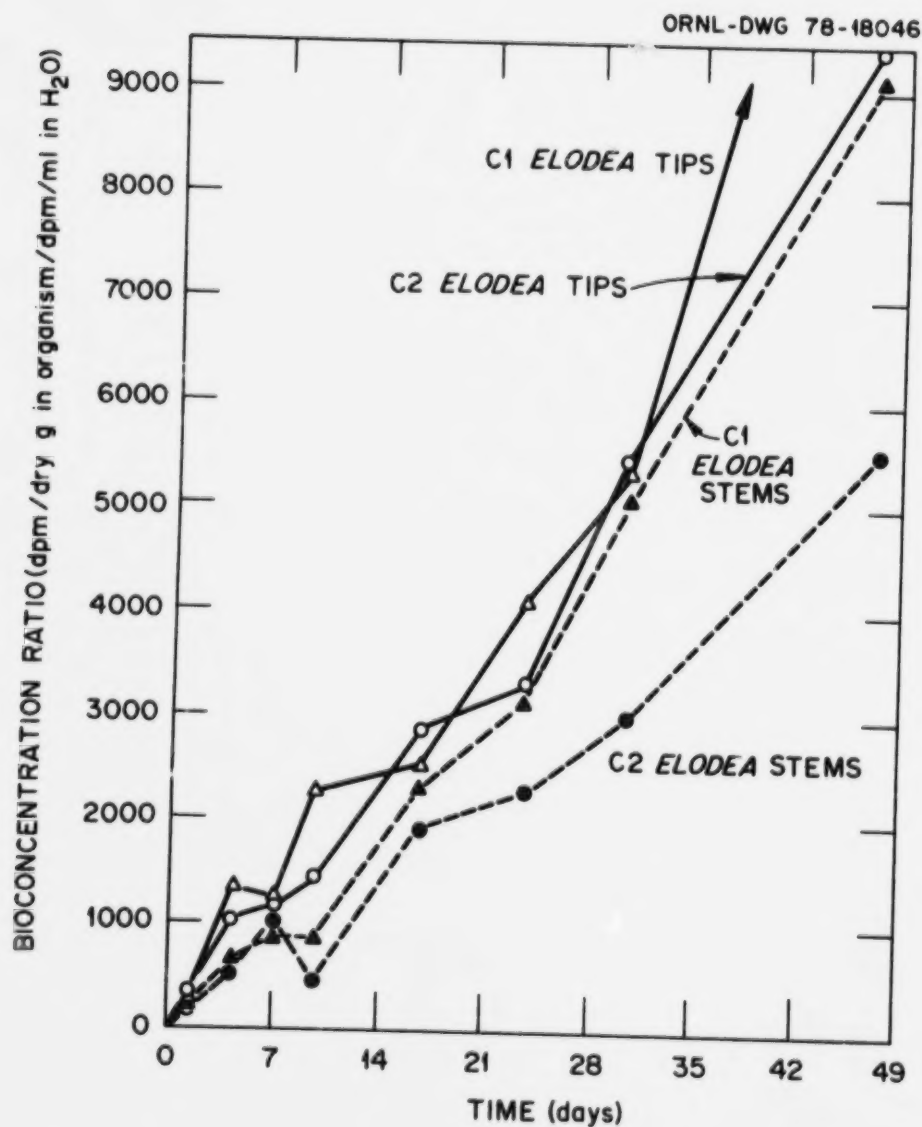


Figure 3.34. Bioaccumulation ratios for ^{203}Hg in Elodea tips and stems, Experiment 7. C1 and C2 are the two replicates.

ORNL - DWG 78-18045

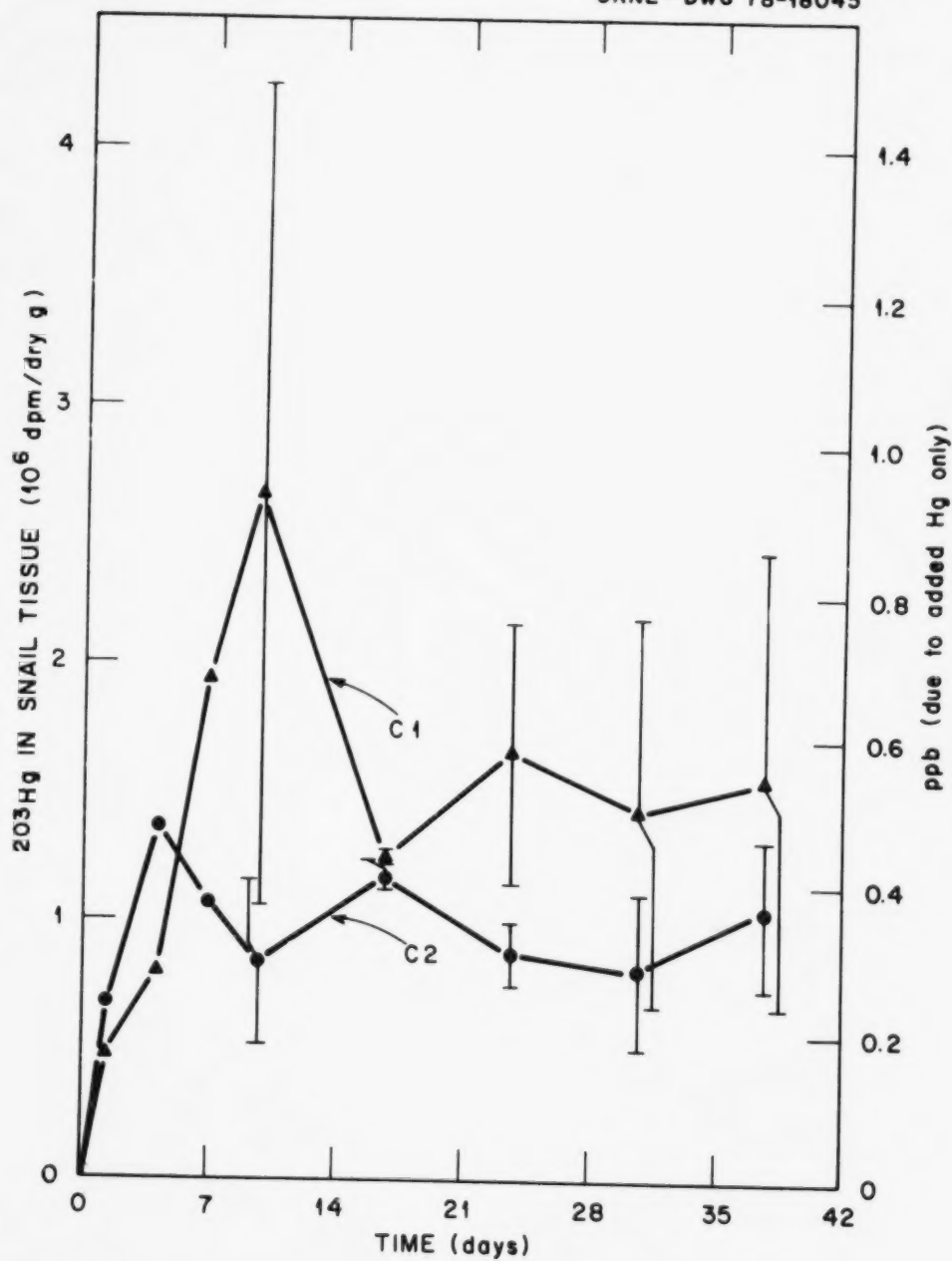


Figure 3.35. Snail tissue ^{203}Hg concentrations, Experiment 7. C1 and C2 are the two replicates. Bars around each point indicate 1 S.D.

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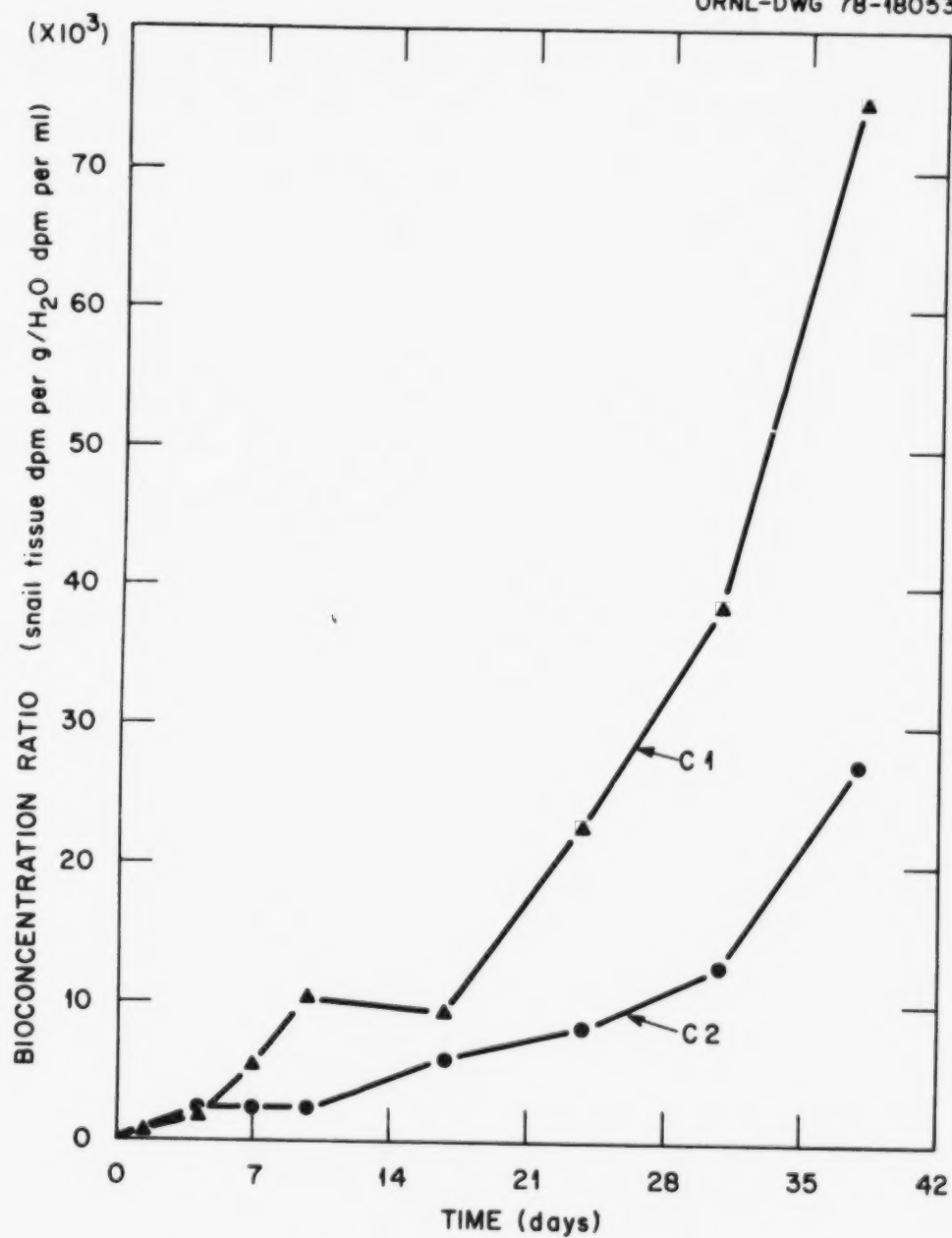


Figure 3.36. Bioaccumulation ratios for ^{203}Hg in snail tissue, Experiment 7.

Table 3.23 shows the final distribution of the Hg addendum among the several components of each microcosm. Nearly 25% of Hg introduced into C1 could not be accounted for with certainty at the end of the experiment; 32% was missing in C2. Sediment sampling error, methylation and subsequent volatilization of Hg, and adsorption onto glass walls of the microcosms probably account for most of the missing Hg. In any event, about 90% of Hg remaining in both systems was in the sediment, 7 to 8% in macrophytes, 1.5% in water, and about 0.5% in snails.

Zooplankton, primarily cladocerans, occurred in numbers large enough to sample in microcosm C1 only. Zooplankton concentrated ^{203}Hg with remarkable rapidity, having concentrations on a wet basis as high as 4.5×10^6 dpm $^{203}\text{Hg/g}$ or 1.6 ppm at the first sampling after only 21 hr of exposure (Fig. 3.37). This represents a bioconcentration ratio, $[\text{zooplankton}]/[\text{water}]$, of nearly 4500 times. Subsequent samplings revealed a gradual decline in zooplankton Hg burdens, reflecting the trend but not the rate observed in the water itself. Mercury concentrations in zooplankton had dropped to about 0.24 ppm (680,000 dpm/wet g) at the last sampling, 37 days following introduction of Hg into the water. It should again be noted that the above concentrations are on a wet-weight basis and that a concentration of 1.6 ppm of Hg would indicate a figure 5 to 10 times higher on a dry-weight basis (i.e., 8-16 ppm). It would not be surprising should such high concentrations in zooplankton prove hazardous to some of their natural predators, particularly planktivorous fish, if not to the zooplankton populations themselves.

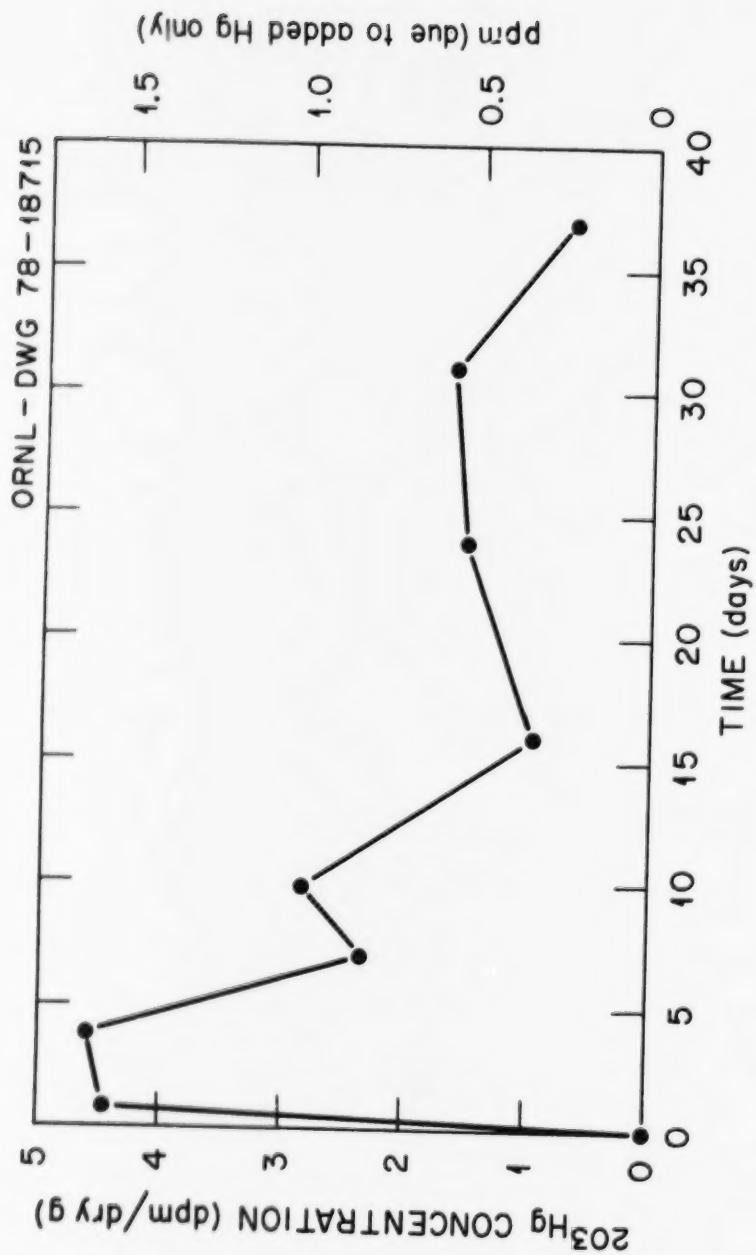


Figure 3.37. Zooplankton ^{203}Hg concentrations, Experiment 7, replicate C1.

3.2.2.5 Anthracene

Aqueous effluents from coal conversion facilities are expected to include hundreds or thousands of organic compounds, including phenols, aromatic amines, monoaromatic hydrocarbons, thiophenes, and polycyclic aromatic hydrocarbons (PAH) (Herbes et al. 1976). PAH are of particular concern for several reasons: (a) many are carcinogenic; (b) PAH may not be removed efficiently by biological waste treatment; (c) some appear to be resistant to degradation; and (d) their environmental fate is practically unknown (Herbes et al. 1976).

We selected anthracene, a 3-ring PAH, for intense study as a representative compound of this class. Anthracene is intermediate in size (M. W. = 188), its solubility in water is 73 $\mu\text{g/liter}$ (Mackay and Shin 1977), and its octanol:water partitioning coefficient is 2×10^4 (Leo 1975). The characteristics of anthracene which determine its fate in aquatic environments have been summarized by Southworth (1977). Anthracene is moderately volatile, with a predicted volatilization half-life of 300 hr in a 1-m deep water column under quiescent conditions. It sorbs strongly to suspended particulate organic matter (K_d = concentration on particulates/concentration in water = 25,000), an order of magnitude less to clay particles (K_d = 1600), and only slightly to silt particles (K_d = 100) (Southworth 1977). Anthracene is rapidly degraded in sunlight. The photolytic half-life in a 2-cm column of distilled water is approximately 35 min under midday, midsummer sunlight at 35°N. Because photolysis is a function of light intensity, adsorption of light by the water would probably limit photolysis to the upper 100 cm in most natural waters (Southworth 1977). Hydrolysis of anthracene has not been found to occur.

Microbial degradation of anthracene has been measured in short-term radiotracer experiments. A mixed culture of microbial strains derived from soil around an oil-drilling site rapidly converted anthracene to polar compounds, with over 90% conversion within 90 min (Herbes et al. 1977). In samples of sediment from an oil-contaminated stream, anthracene was readily broken down (half-life = 12 days), but only 10% of the radiotracer appeared in the polar fraction. In sediment from an uncontaminated stream, the degradation rate was an order of magnitude slower (Herbes and Schwall 1978).

As explained above (Section 3.1.2), this experiment went beyond the previous experiments by examining the chemical fate of the test contaminant in addition to its distribution among microcosm components. This was possible because analytical procedures had been developed as part of other programs in the Environmental Sciences Division. This experiment has been described by Giddings et al. (in press), from which the following sections are taken.

Materials and Methods

Two 6-month-old microcosms were treated with $[9-^{14}\text{C}]$ anthracene (specific activity = 32 $\mu\text{Ci}/\text{M}$, Amersham/Searle) in July 1977. Approximately 1 μCi of anthracene was dissolved in 20 ml of acetone and diluted to 500 ml with distilled water. The solution was divided into two 250-ml aliquots. One milliliter of each aliquot was withdrawn for radioassay, and the remainder was mixed with about one liter of microcosm water and slowly poured into the microcosm. The initial concentration of anthracene in the water was calculated to be 0.5 $\mu\text{g}/\text{liter}$.

Two 1-ml water samples were removed from each microcosm, two to five times weekly, placed in a scintillation vial with 10 ml of Bray's solution, and counted on a Packard Tri-Carb Liquid Scintillation Spectrometer. Samples of sediment, Elodea, and filamentous algae were taken approximately once a week. Only the upper 1 cm of sediment was collected, except for the final samples in which entire cores were removed and sectioned. Samples of sediment and organisms were dried overnight at 55°C, weighed, oxidized with a Packard Tri-Carb Sample Oxidizer, and counted.

The final samples were taken 70 to 84 days after anthracene addition. In addition to the samples described above, periphyton (from the aquarium walls), snails, snail eggs, zooplankton, and water mites were collected. The snails were removed from their shells, and all samples were then dried and oxidized as described above.

Water samples for extraction and thin-layer chromatography (TLC) were taken from the microcosms 11 times during the course of the experiment. Sediment samples for extraction were taken at the end of the experiment; only the upper 2 cm were used. Algae samples were removed and extracted on Day 42. Daphnia magna were abundant at the time of anthracene addition, and 10 to 12 individuals were removed daily until the population was depleted.

Samples were extracted as follows: (1) Water samples were acidified with a mineral acid and extracted twice with ethyl acetate in a 1:5 ratio of solvent to water. For each extraction, solvent-water mixtures were agitated for 45 min on a wrist-action shaker. (2) Wet sediment samples were extracted overnight with acetone in a Soxhlet

apparatus. Acetone was evaporated from the extracts, and the remaining liquid was extracted three times with equal volumes of ethyl acetate.

(3) Excess water was blotted from Daphnia and algae. Samples were weighed, homogenized in acetone, and centrifuged.

The extracts from each sample were pooled, dried over anhydrous calcium sulfate, centrifuged, and reduced to 2 ml under nitrogen. One milliliter of the extract was counted by liquid scintillation spectrometry to determine total extractable ^{14}C , and the remainder of the extract was subjected to thin-layer chromatography followed by radioautography. Toluene was used as a solvent for thin-layer chromatography on silica gel 60 TLC plates. Radioautograms were prepared with x-ray no-screen film.

Residues remaining after extraction of algae and sediment were oxidized to determine unextractable radioactivity.

Results

The ^{14}C activity in the water declined rapidly for the first 15 days, then declined more slowly for 50 days thereafter (Fig. 3.38). The mean first-order kinetic rate constant was 0.055/day for Days 0-15, and 0.007/day for Days 20-65. There was a suggestion of accelerated ^{14}C loss over the last 20 days of the experiment.

Table 3.25 shows the ^{14}C activity in all microcosm components 12 weeks after anthracene was added. Approximately 30 cpm/ml (15% of the initial ^{14}C) remained in the water. Most of the ^{14}C accumulated in the upper 2 cm of sediment, with much lower activity below this level. The total activity in the sediment was estimated to be 10.2×10^6 and

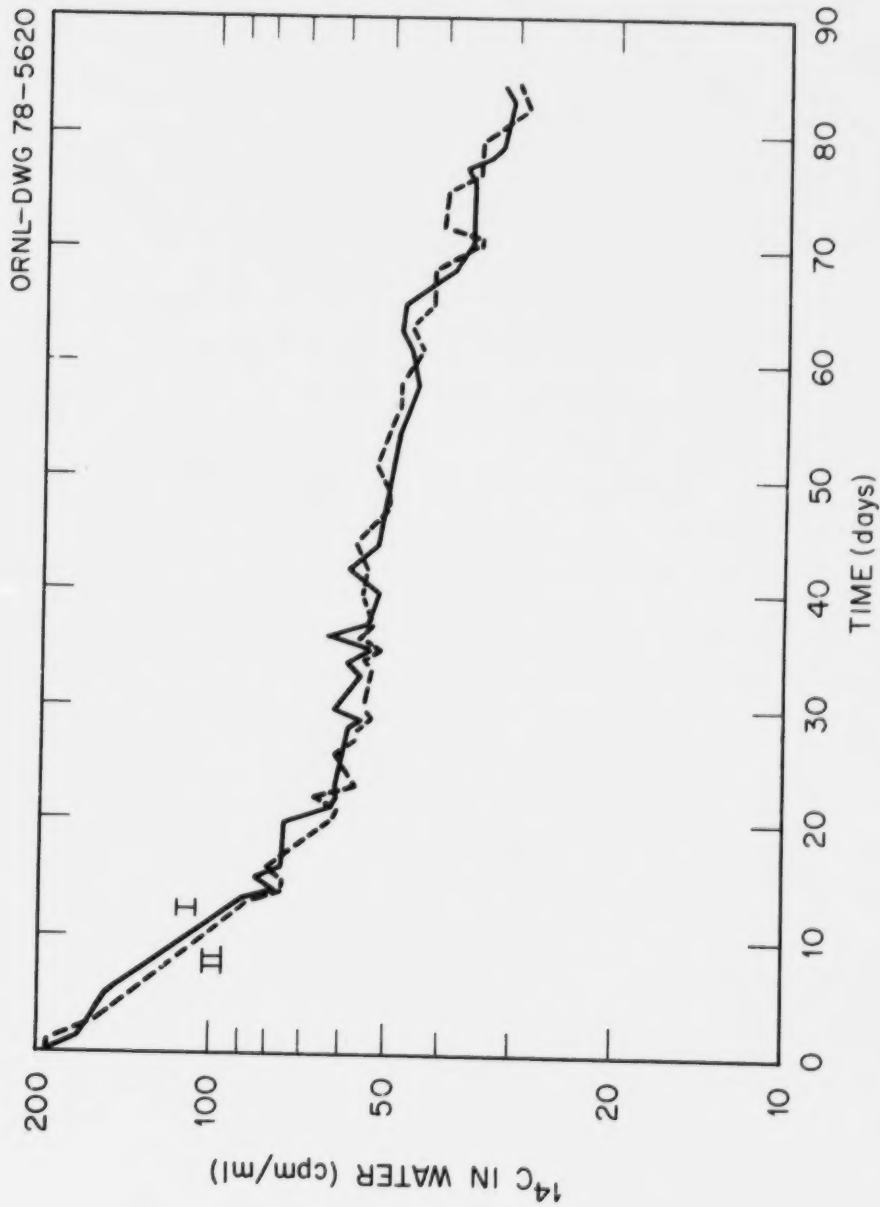


Figure 3.38. ^{14}C activity in microcosm water, Experiment 8. I and II are the two replicates.

Table 3.25. Final ^{14}C activity in microcosm components twelve weeks after addition of 10.4×10^6 cpm as anthracene

Component	M1	M2
	(cpm/g dry wt)	
Water ^a	31.6	30.5
Sediment		
Upper 2 cm	7,220	6,080
Lower 5 cm	875	374
Plants		
Elodea	25,800	28,000
Algae (benthic)	22,500	29,800
Periphyton	17,800	20,100
Animals		
Zooplankton	12,400	26,700
Water mites	31,900	45,700
Snails (tissue)	30,100	56,800
Snails (eggs)	21,300	21,600

^aWater activity in cpm/ml.

8.0×10^6 cpm for the two replicate microcosms. The activity in the water and the sediment accounted for 92 to 114% of the ^{14}C initially added (Table 3.26). It is therefore unlikely that volatilization of ^{14}C compounds was significant in this experiment.

All organisms accumulated activity to about 10^3 times the activity in the water. In each microcosm, the highest activities were found in water mites and snail tissue. These animals probably represent the ends of the grazing and detrital food chains, respectively, implying that anthracene or its degradation products may undergo biomagnification (accumulation up the food chain).

Chromatographic analysis of water, sediment, Daphnia, and algal extracts showed the presence of anthracene, four radiolabelled derivatives, and an unresolved polar fraction (Table 3.27). Co-chromatography of sample extracts with internal nonradioactive standards permitted identification of anthracene ($R_f = 0.84$); however, R_f 's of 9-anthraldehyde ($R_f = 0.40$), anthraquinone ($R_f = 0.31$), and anthracene-9-carboxylic acid ($R_f = 0.01$) did not agree with R_f values of radioactive unknowns. The contribution of each compound to total extractable activity in the water during the experiment can be seen in Fig. 3.39. Only trace amounts of anthracene were recovered from the water after Day 20. The disappearance of Compound 5 closely followed the disappearance of anthracene, suggesting that anthracene was converted directly to Compound 5, which was not persistent. Compound 3 was present in large quantity throughout the experiment. This compound constituted as much as 49% of the extractable radioactivity in the water (Day 28), and although its relative abundance decreased

Table 3.26. ^{14}C budget

	Anthracene microcosms	
	I (cpm)	II (cpm)
Added	10.4×10^6	10.4×10^6
Recovered		
Water	1.7×10^6	1.6×10^6
Sediment	10.2×10^6	8.0×10^6
Total	11.9×10^6	9.6×10^6
% recovered	114	92

Table 3.27. Percentage distribution of radioactivity in extracts

Compound	R	Day 6		Day 42		Day 84	
		<u>Daphnia</u>	Water	Algae	Water	Sediment	Water
Anthracene	0.84	22.3	9.4	1.5	0.9	32.1	-
5	0.76	19.3	5.6	1.6	0.8	15.7	-
4	0.34	-	-	-	-	-	-
3	0.27	22.7	39.5	40.5	24.7	29.5	13.1
2	0.08	-	4.9	-	-	-	-
1	0.04	-	-	1.9	2.0	6.7	2.4
Polar	0.00	35.7	40.6	54.5	71.7	16.0	84.5

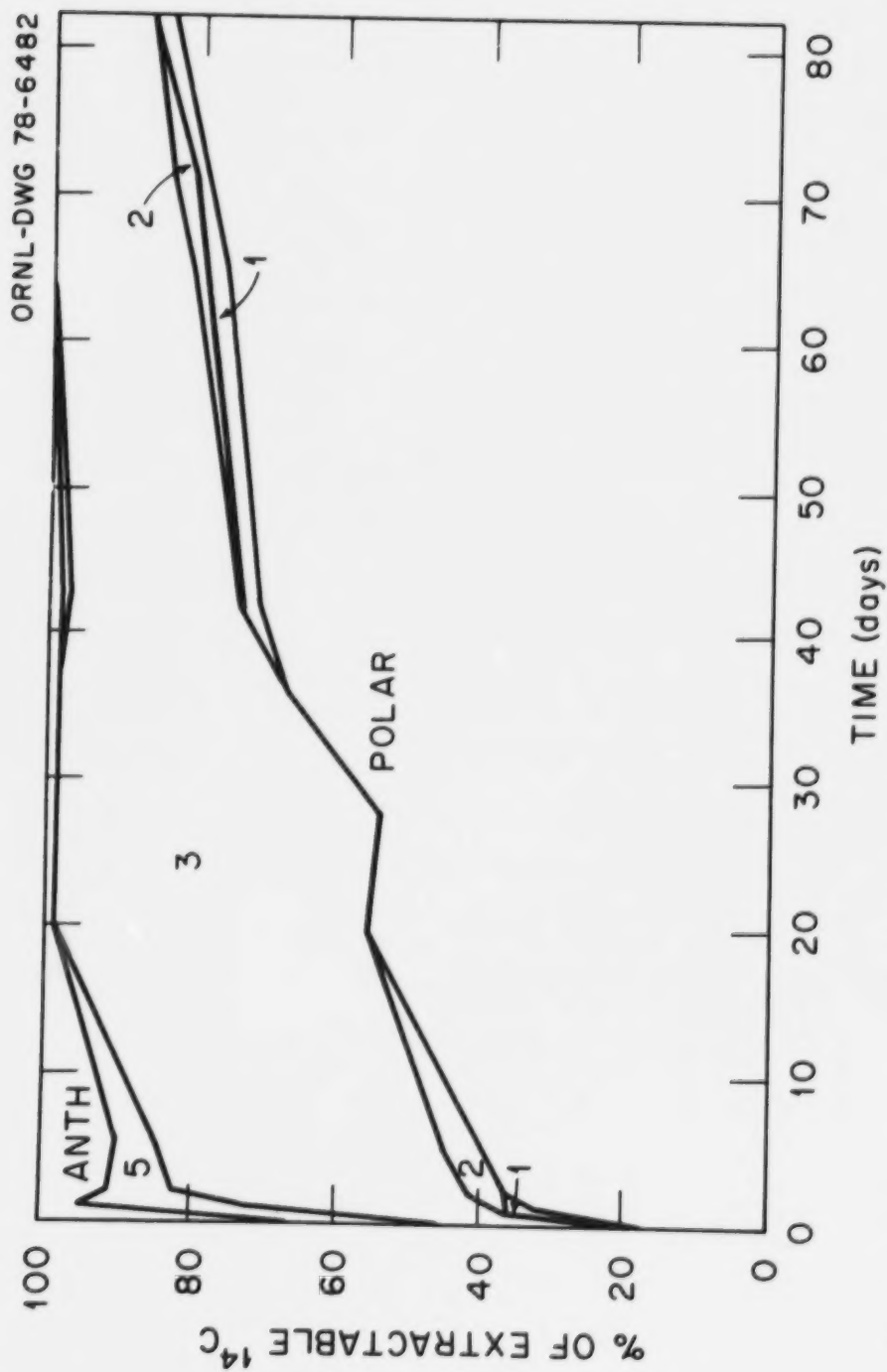


Figure 3.39. Distribution of ^{14}C activity in extracts from microcosm water, Experiment 8. Compounds numbered as in Table 3.27. Data are averages from two replicate microcosms.

thereafter, it was still present when the experiment was terminated on Day 84. Forty-five percent of the total ^{14}C in the water was not extractable on Day 84.

Distilled water to which $[9\text{-}^{14}\text{C}]$ anthracene was added yielded anthracene, a polar fraction, the four derivatives found in microcosm extracts, and an additional compound with $R_f = 0.34$, after 7 days in the environmental chamber. The latter compound, which constituted 14% of the radioactivity extracted from the distilled water, was never detected in microcosm extracts. Figure 3.40 is a comparison of extractable fractions from microcosm water and from distilled water. After seven days, the ^{14}C profile from distilled water was very similar to that of microcosm water on Day 12. Anthracene was nearly absent from both systems. The large polar fraction recovered from distilled water (45%) as well as from microcosm water (48%) is particularly striking since it indicates that anthracene is readily degraded to polar compounds in the absence of microcosm biota. In studies of abiotic degradation of anthracene, Southworth (1977) found that photolysis was rapid but hydrolytic breakdown was slow; therefore, the anthracene transformation products detected in the microcosm water and in the distilled water were probably due to photolysis.

After 84 days, unextractable ^{14}C accounted for 40% of the activity in the upper 2 cm of sediment and 30% of the activity in the lower sediment. At least a part of this ^{14}C was probably incorporated into bacterial cells. One-third of the extractable activity, or 17% of the initial addition, was anthracene, and another large fraction was Compound 5 (Table 3.27). The polar fraction was only 16% of the

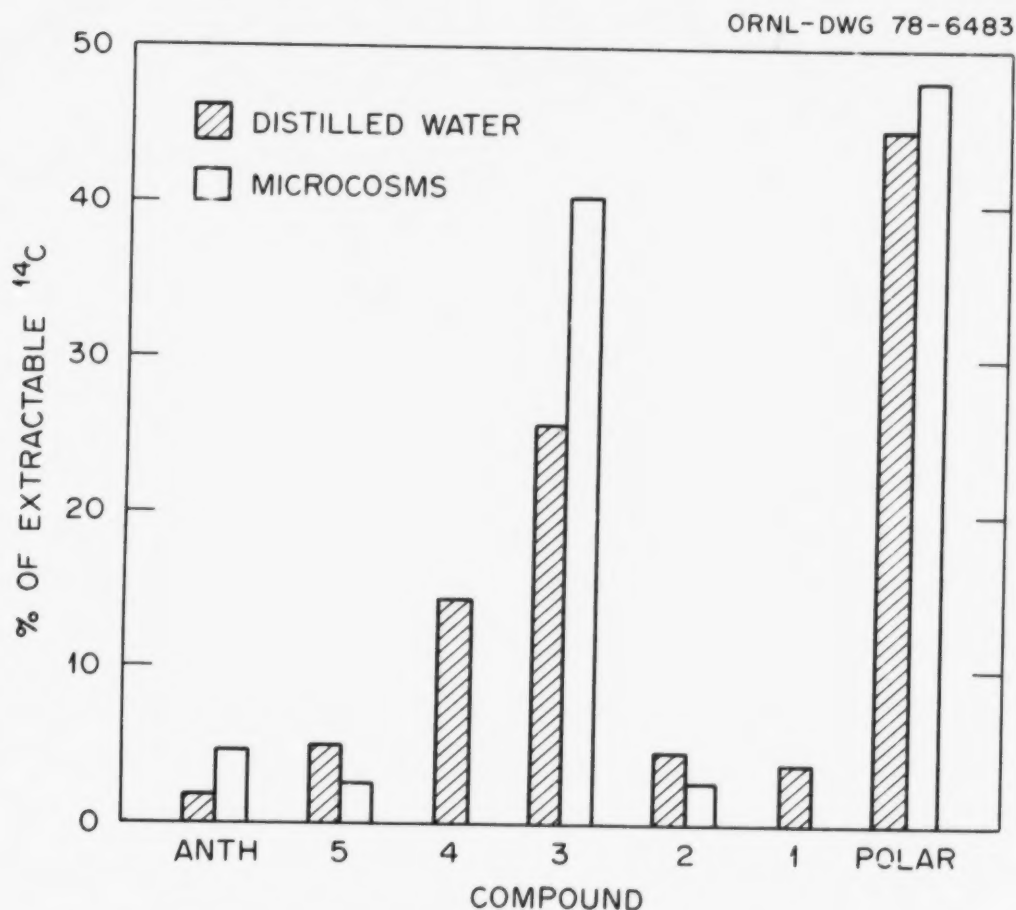


Figure 3.40. Comparison of ^{14}C distribution in extracts from microcosm water and distilled water, Experiment 8. Distilled water was extracted 7 days after anthracene addition. Microcosm data for Day 12 were interpolated from Fig. 3.39. Compounds are numbered as in Table 3.27.

extractable activity. These findings are consistent with observations of Herbes and Schwall (1978). The data imply that microorganisms in the sediment do not readily degrade anthracene, and that anthracene in the sediment could provide a source for continued exposure of benthic organisms to the parent compound.

Daphnia was found to contain higher levels of extractable anthracene than those found in water (Table 3.27). The distribution of activity in extracts of algae was not markedly different from the distribution in water extracts taken at the same time, except that more of Compound 3 was present (Table 3.27). Sixty-five percent of the ^{14}C in the algae was unextractable.

Conclusions

(1) Anthracene disappeared rapidly from the water. After 84 days, 17% of the anthracene initially added was recovered unchanged in the sediment. Approximately 40% was degraded to more polar compounds, apparently by photolysis. An additional 35% was unextractable from the sediment, probably reflecting incorporation into bacterial cells, and 7% was transformed into an unextractable form in the water.

(2) Several of the degradation products persisted in the water or the sediment for the duration of the experiment. The most abundant product (Compound 3) was found in all microcosm components examined. Compound 4, one of the main degradation products formed in distilled water exposed to light, was never found in the microcosms, implying that it was broken down further by the microcosm biota.

(3) Anthracene was much less susceptible to degradation when sorbed to sediment than when dissolved in water. Since sorption occurs rapidly in shallow water, a significant fraction of the anthracene entering a pond or stream would accumulate in the sediment. This would probably be available for uptake by organisms interacting with sediments (Walter and Johnson 1977).

3.2.3 Ecological Effects (J. M. Giddings)

Although the primary goal of this project was to develop microcosms for testing contaminant transport and fate, we recognized that our pond microcosms could also be used to test contaminants for their effects on whole ecosystems. One obstacle to the inclusion of ecosystem-level effects in a testing program is the lack of readily measurable indicators of contaminant-induced stress. This methodological deficiency should not obscure the basic importance of studies at the ecosystem level, since disruptions of ecosystem functions may have far-reaching effects that are not predictable from single-species toxicity tests. We sought to identify ecosystem-level effects of contaminants by monitoring ecosystem metabolism and nutrient pools in treated and untreated microcosms. Our objective was to locate "pulse points," parameters which could be easily monitored in microcosms to indicate ecosystem dysfunction. The following discussion is based on results of Experiment 2, in which replicate microcosms were treated with sodium arsenate at 0, 0.066, 11.5, and 143 ppm.

3.2.3.1 Production/Respiration Ratios

The ratio of primary production to total community respiration (P/R) is an integrative index of ecosystem metabolism which can be easily measured in many aquatic ecosystems. Odum (1956) proposed the use of P/R for classifying ecosystems as autotrophic ($P/R > 1$) or heterotrophic ($P/R < 1$), and noted that either type of system tends to approach $P/R = 1$ over time. Odum (1969) listed $P/R = 1$ as an attribute of mature ecosystems, concluding that the ratio could be used as an index of relative maturity. P/R ratios of approximately 1 have been found in many aquatic ecosystems (Riley 1956, Odum 1957, Odum and Hoskins 1958, Jordan and Likens 1975).

In studies with aquatic microcosms, P/R was found to depart from 1 when a system was disturbed. This effect was observed whether the disturbance was in the form of temperature stress (Beyers 1962), light reduction (Copeland 1965), increased grazing (McConnell 1962, Beyers 1963), or toxicants (Whitworth and Lane 1969). Thus it appears that P/R is a sensitive indicator of stress-induced changes in ecosystem metabolism. We tested the effects of arsenic on the P/R ratio as part of Experiment 2.

Net primary production and total ecosystem respiration are shown in Fig. 3.41. Before treatment with As, the mean net primary production for all 12 microcosms increased gradually from 3.7 ppm D.O./12 hr at week 6 (S.D. = 0.5) to 4.6 ppm/12 hr at week 10 (S.D. = 0.7). This trend continued in the control and the 0.066-ppm microcosms, reaching 5.4 (S.D. = 0.5) at week 16. The two higher As concentrations, however, resulted in immediate reductions in net primary production. In the

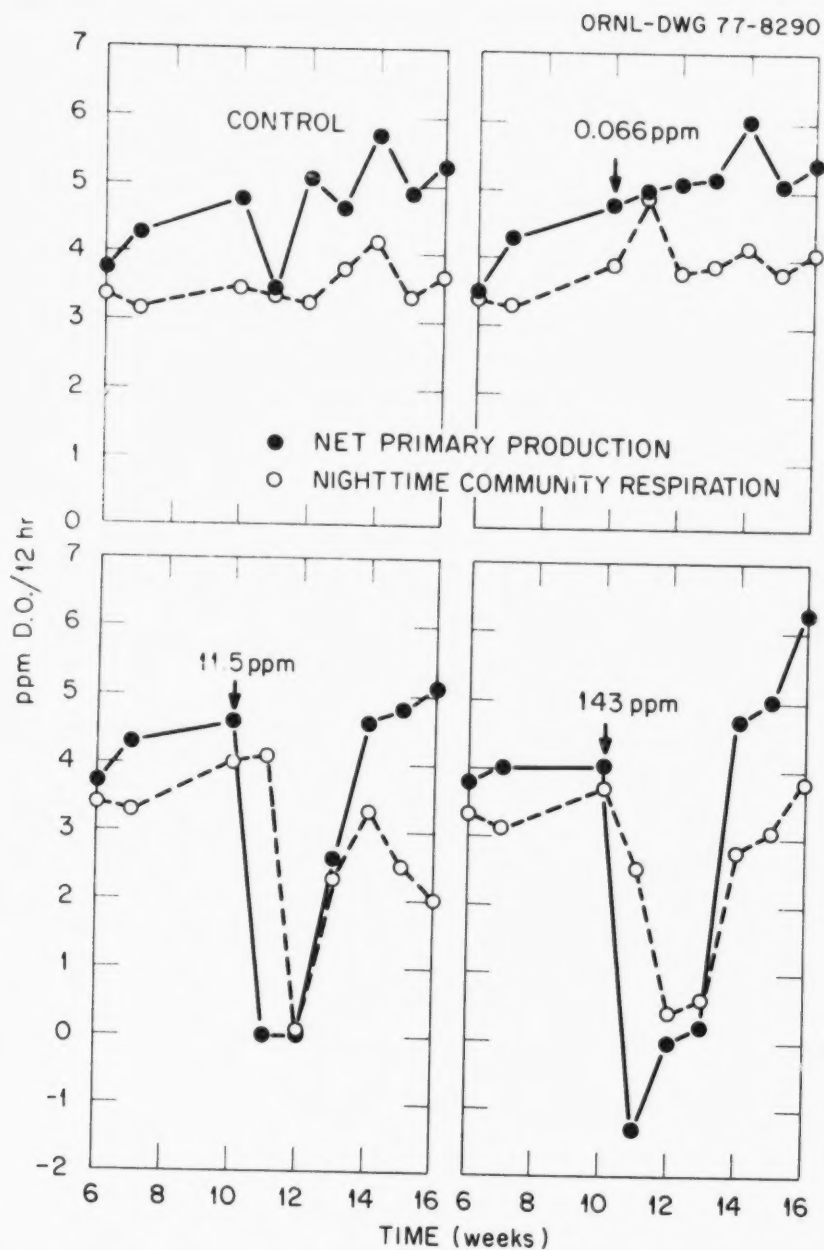


Figure 3.41. Net production and nighttime community respiration in microcosms at three arsenic levels, Experiment 2. Open circles: nighttime community respiration; filled circles: net production. Each point represents the mean of three microcosms. Arsenic was added, in the concentrations indicated, immediately following P and R measurement at week 10.

143-ppm microcosms, dissolved O_2 concentrations declined during the light period one week after treatment (i.e., gross photosynthesis was less than daytime community respiration in these microcosms). Net production remained near zero for two weeks in the 11.5-ppm microcosms, and for three weeks in the 143-ppm microcosms. Toward the end of the experiment, productivity recovered in both treatments; in fact, the net production at week 16 in the 143-ppm microcosms was considerably higher than that in the control. Recovery was accompanied by shifts in community structure. Macrophytes were replaced by Spirogyra in the 11.5-ppm microcosms, and by blue-green algae in the 143-ppm microcosms.

The effects of 11.5 and 143 ppm As on nighttime community respiration were similar to, but less drastic than, effects on production. The decline in respiration was about one week behind the decline in productivity, and recovery was also slower. Respiration declined toward the end of the experiment in the 11.5-ppm microcosms; this effect is unexplained. Respiration increased very slightly during the experiment in the control and 0.066-ppm microcosms, averaging 3.4 and 3.9 ppm $D.O./12$ hr at week 6 and week 16, respectively.

The ratio of net primary production to nighttime community respiration averaged 1.2 for all microcosms before As treatment. The ratio remained fairly constant (1.0 to 1.5, mean 1.4) in the control and 0.066-ppm microcosms throughout the experiment (Fig. 3.42). In the 11.5- and 143-ppm microcosms, P/R dropped to zero or below for two weeks following As addition. The decline was greater at 143 ppm than at 11.5 ppm. By the third week after As treatment P/R increased, even in the 143-ppm microcosms where net production remained very low for an

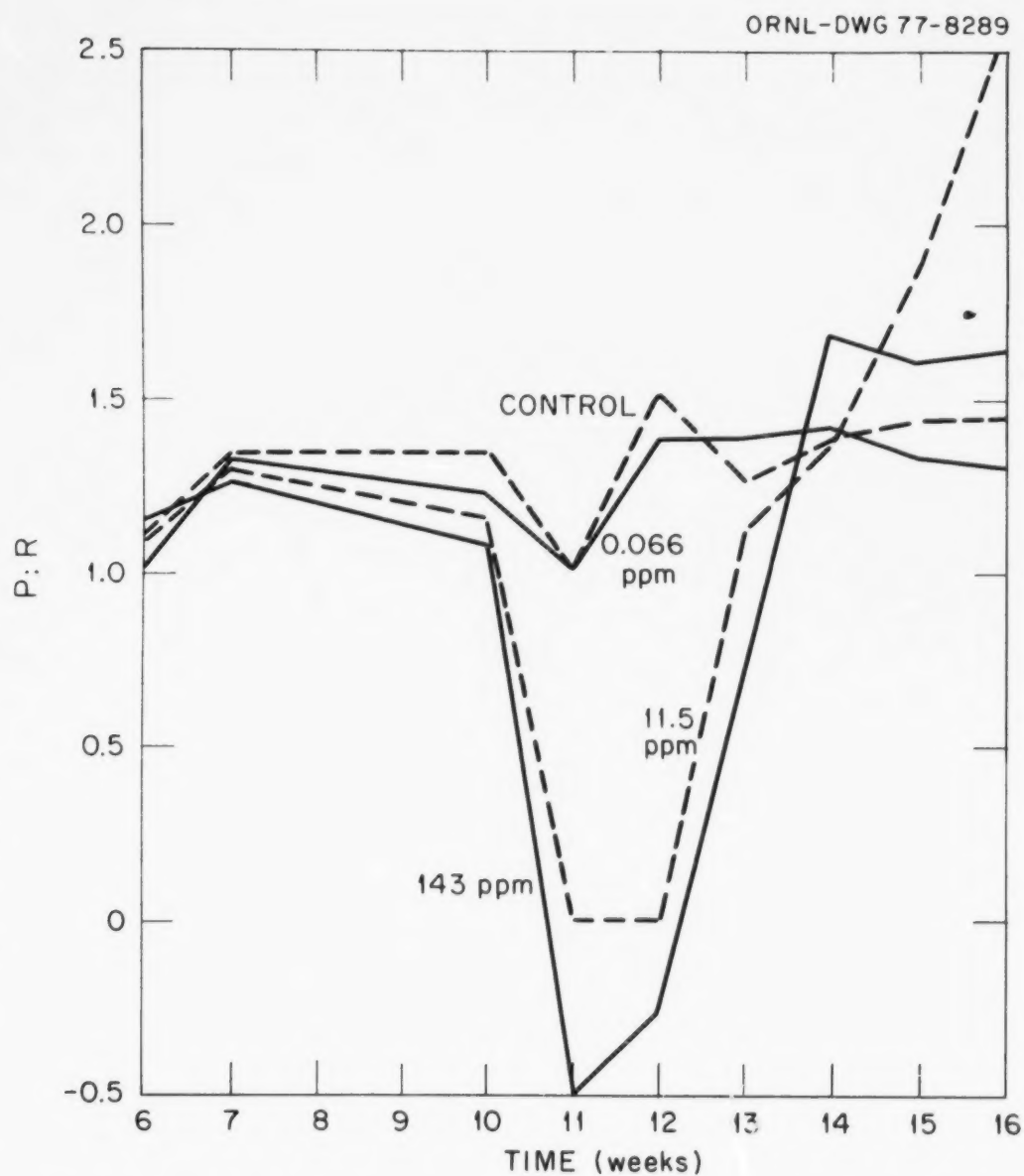


Figure 3.42. Production/respiration ratios in microcosms at three arsenic levels, Experiment 2. Each point represents the ratio of mean net production to mean nighttime community respiration for three microcosms. Arsenic was added during week 10.

additional week. The highest P/R ratios recorded in the entire experiment were in the 11.5-ppm microcosms at week 16 ($P/R = 2.5$).

The P/R recovery in both the 11.5- and 143-ppm treatments was similar to that observed by Copeland (1965) in marine microcosms exposed to a reduction in light intensity. In both cases, recovery was accompanied by changes in community structure. The ability to compensate for stress by species shifts is an important property of ecosystems. Until the theoretical relations between population dynamics and ecosystem dynamics are more fully understood, it will be difficult to predict the effects of a particular stress on a particular ecosystem. Certainly the P/R response of these microcosms to As treatment could not have been predicted by measuring the responses of the separate components.

While further refinement of this technique is necessary, the P/R ratio appears to be a useful indicator of ecosystem response to stress. The lowest treatment in this experiment had no effect on the P/R ratio of the microcosms, while higher concentrations produced progressively greater reductions in P/R. The As concentrations used here were apparently at the extremes of the dose-response curve, but the method appears usable for determining the response of an aquatic ecosystem to different concentrations of a contaminant.

We attempted to test this hypothesis in Experiment 4. However, P/R was highly variable between replicates in this experiment, which we believe was due to the disturbing effect of monitoring dissolved oxygen in the 7-liter microcosms. Because of the inconsistency of the results, no clear trends in P/R were discernable. Further research is needed in this area.

3.2.3.2 Water Chemistry

Effects of contaminant stress were evident in nearly every chemical parameter monitored in Experiment 2. Declines in the P/R ratio at 11.5 ppm and 143 ppm arsenic (Fig. 3.42) resulted in dramatic changes in pH (Fig. 3.43) and dissolved oxygen (Fig. 3.44). These two parameters are clearly under biological control in the microcosms. Since pH and D.O. are more easily measured than P/R, they might be used as indirect indicators of contaminant effects on ecosystem metabolism.

Concurrently with the drop in pH, alkalinity increased in the 11.5- and 143-ppm microcosms (Fig. 3.45), probably due to dissolution of carbonates which had precipitated during the first two months of the experiment. This conclusion is supported by the significant increases in Ca, K, and Mg in these microcosms at the end of the experiment (Table 3.28). Sodium concentrations also rose, but the increases corresponded to the amount added as sodium arsenate. Concentrations of Cl and SO_4 were also significantly higher in the microcosms receiving high arsenic doses.

Significant increases in dissolved organic carbon were noted in the 11.5-ppm microcosms one week after treatment, and in the 143-ppm microcosms three weeks after treatment (Fig. 3.46). These increases might represent excretion and/or autolytic release of DOC from plants and animals, or temporary inhibition of DOC utilization by bacteria. DOC concentrations returned to the same level as the controls within one to two weeks.

Concentrations of NO_3 and NH_4 showed a tendency to increase, following treatment with 11.5 or 143 ppm arsenic (Table 3.29), but the

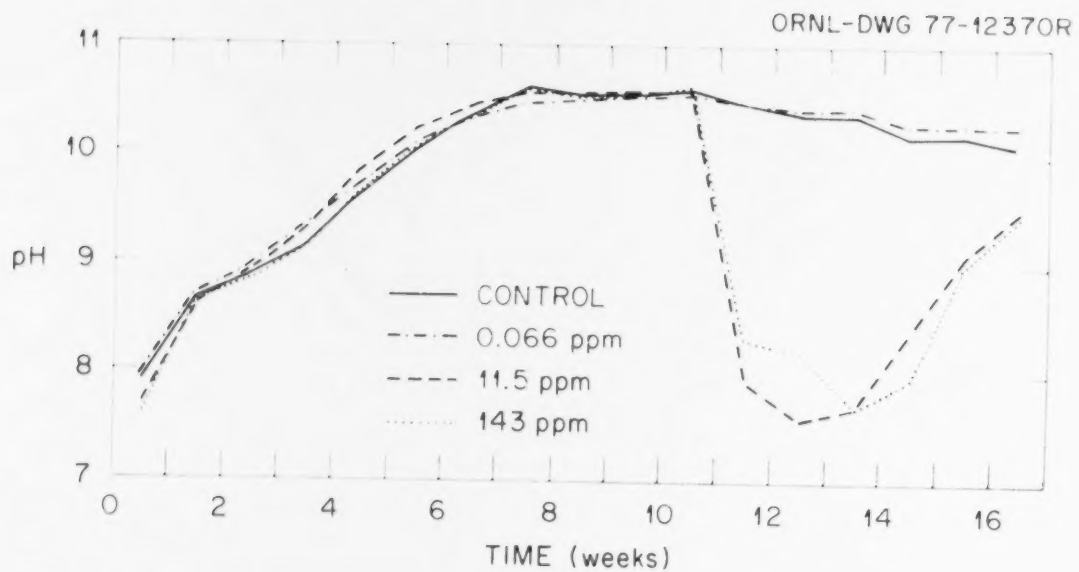


Figure 3.43. pH, Experiment 2. Each line represents the mean of three replicate microcosms. Arsenic was added during week 10.

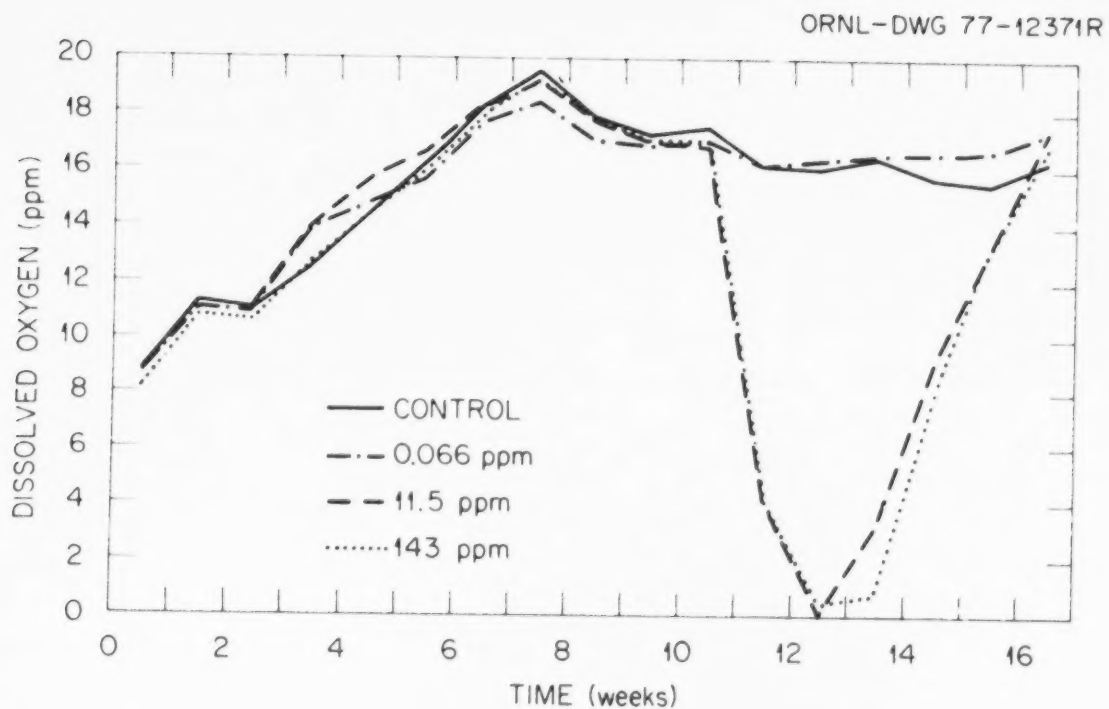


Figure 3.44. Dissolved oxygen concentrations, Experiment 2. Each line represents the mean of three replicate microcosms. Arsenic was added during week 10.

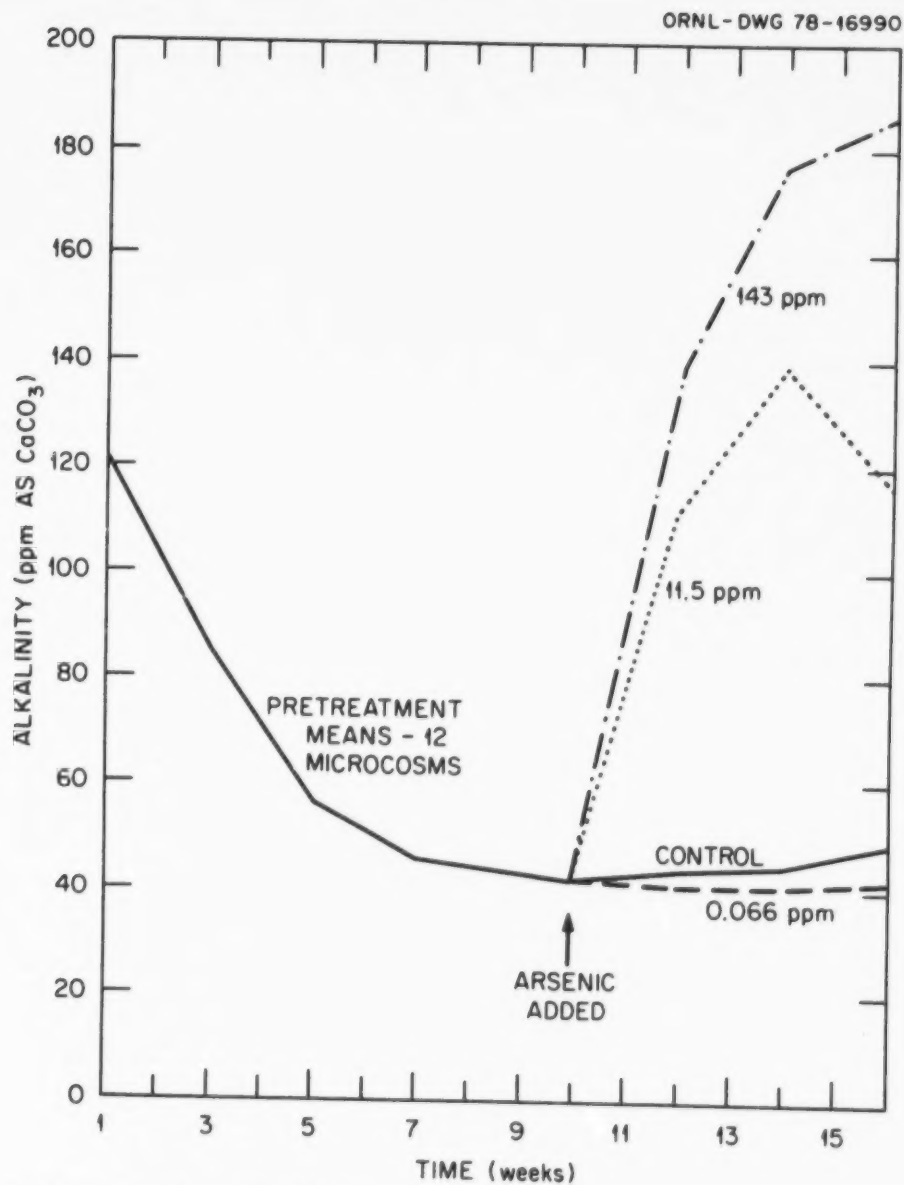


Figure 3.45. Alkalinity, Experiment 2. The line from week 1 to week 10 represents the mean of 12 replicate microcosms; lines after week 10 represent the means of three replicate microcosms. Arsenic was added at the point indicated. Alkalinity is expressed in ppm as CaCO_3 .

Table 3.28. Effects of arsenic on microcosm water chemistry:
Experiment 2^a

	Initial (ppm)	Final (ppm)			
		Control	0.066 ppm As	11.5 ppm As	143 ppm As
Na	0.74	0.03	0.03	8.25	61.17
	(0.06)	(0.02)	(0.02)	(1.00)	(1.60)
K	5.56	0.10	0.10	1.87	7.27
	(0.55)	(0.0)	(0.0)	(1.86)	(0.23)
Ca	30.51	11.23	10.63	19.13	32.13
	(2.49)	(0.47)	(1.44)	(8.20)	(1.33)
Mg	10.13	5.13	3.97	6.17	5.97
	(0.84)	(0.67)	(0.57)	(1.17)	(0.21)
Cl	1.44	0.30	0.28	1.36	2.50
	(0.17)	(0.04)	(0.04)	(0.62)	(0.42)
SO ₄	3.21	3.18	5.36	2.94	8.39
	(0.24)	(2.50)	(1.79)	(0.47)	(0.58)

^aValues are means for three replicates; standard deviations in parentheses.

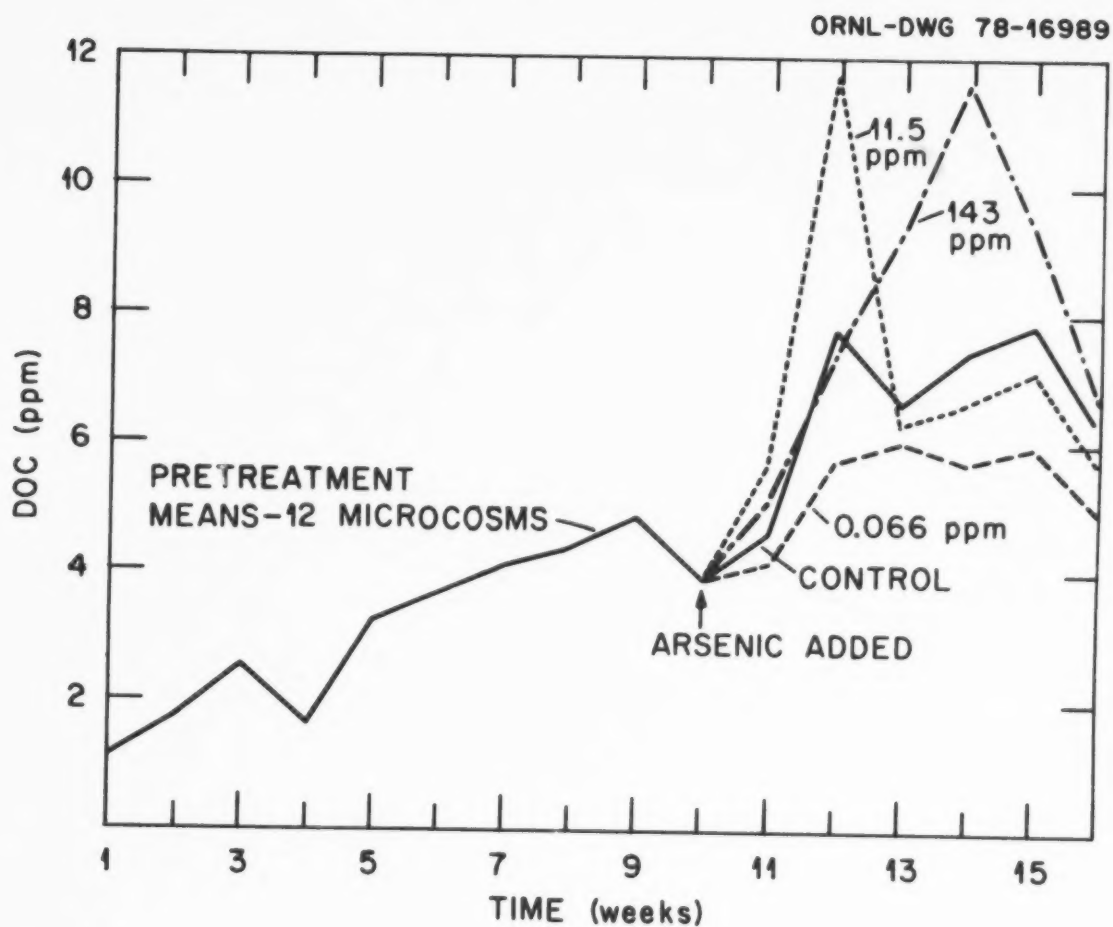


Figure 3.46. Dissolved organic carbon, Experiment 2. Lines as in Fig. 3.45.

Table 3.29. Inorganic nitrogen concentrations following arsenic treatment: Experiment 2^a

Date	Days after As addition	Treatment (ppm As)			
		0	0.066	11.5	143
NO ₃					
9/20/76	3	0.007	0.015	0.010	0.009
9/27	10	0.008	0.012	0.011	0.011
10/4	17	0.018	0.009	0.012	0.012
10/11	24	0.007	0.017	0.009	0.005
10/18	31	0.017	0.014	0.049	0.011
10/25	38	0.006	0.012	0.006	0.002
11/1	45	0.006	0.007	0.007	0.004
NH ₄					
9/20/76	3	0.013	0.018	0.023	0.015
9/27	10	0.015	0.022	0.034	0.054
10/4	17	0.008	0.004	0.010	0.019
10/11	24	0.013	0.032	0.019	0.052
10/18	31	0.015	0.009	0.024	0.026
10/25	38	0.007	0.007	0.019	0.040
11/1	45	0.007	0.006	0.010	0.015

^aAll concentrations in ppm, means for three replicates.

data were too variable for statistical confirmation of this trend (see Section 3.2.1.3., and Table 3.1). Effects on phosphorus concentrations were large enough to be significant ($P < 0.01$) in spite of the variability between replicates (Fig. 3.47). The dramatic increase in total P implies a temporary disturbance of normal phosphorus cycling; a disruption in ecosystem function is evident even though we do not know which component(s) were affected. The usefulness of such "black box" nutrient parameters as microcosm "pulse points" might be limited, however, due to the high variability of nutrient measurements. Nutrients in interstitial water (see Section 3.2.1.1.) may prove to be more sensitive to nutrient cycling disruptions than nutrients in the overlying water.

Many of the effects of arsenic on microcosm water chemistry were also observed in pond enclosures (Section 3.2.1.2., Table 3.1). Nutrient concentrations in the enclosures were quite variable, and consistent trends such as those shown in Figs. 3.21 and 3.22 were not found.

3.3 DISCUSSION

3.3.1 Protocol for the Construction of Pond Microcosms (J. M. Giddings)

The following protocol describes the basic procedure for constructing pond microcosms. Since microcosms designed in the manner described are suitable for a wide range of applications in contaminant research, standard experimental techniques are not defined in the protocol. Specific uses of pond microcosms in contaminant testing are discussed in Section 3.3.2.

ORNL-DWG 78-16988

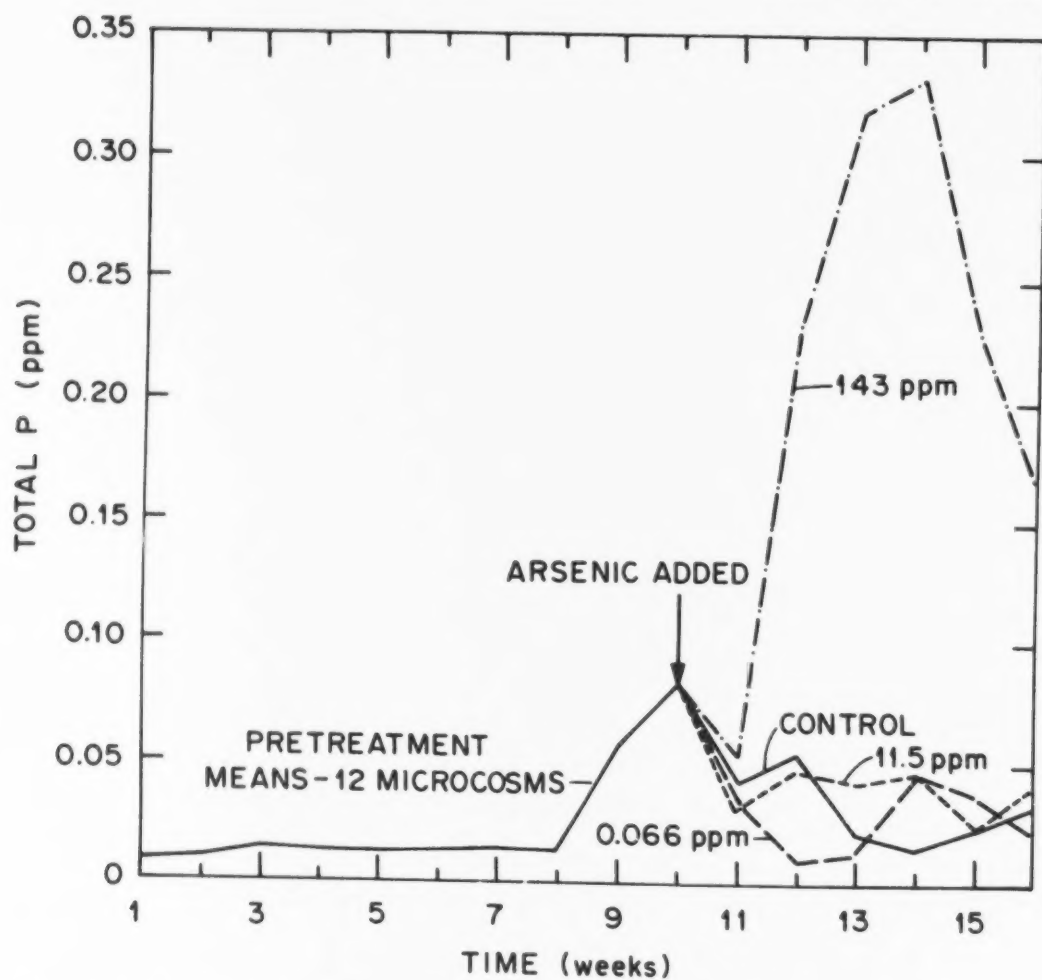


Figure 3.47. Total phosphorus concentrations, Experiment 2. Lines as in Fig. 3.45.

3.3.1.1 Collection of Components

All microcosm components are collected from a single ecosystem, preferably on the same day they are to be used. A shallow pond is the best source of material for pond microcosms, but littoral zones of lakes, or slow-moving rivers, may be acceptable alternatives.

Water is collected first, before the pond has been disturbed by sediment collection. It is sufficient to simply dip the water from the pond with a bucket. At least 60 liters are required for each microcosm.

Sediment is scooped, shoveled, or dredged from the bottom of the pond. Stones, twigs, and other large debris are removed if possible. Ten liters or more of sediment are needed per microcosm.

If a macrophyte community is present, a portion is shoveled from the bottom and placed in a bucket or tub. All of the organisms naturally associated with the macrophyte community can be included, except crayfish, which have a devastating effect on macrophytes in a closed system.

If no macrophyte communities occur in the pond, filamentous algal communities may be collected instead.

The water, sediment, and biota are protected from bright sunlight and extreme temperatures, and placed in an environmental chamber as soon as possible. The temperature of the chamber is set to equal that of the pond water.

3.3.1.2 Assembly of Microcosms

We have found 70-liter slate-bottomed glass aquaria to be convenient microcosm containers. Each aquarium is washed with tap water, rinsed with dilute acid, and rinsed several times with distilled water.

The aquaria are positioned in the environmental chamber before filling, since they are virtually unmoveable when full.

A 5-cm layer of sediment is placed in each aquarium. If replicate microcosms are to be constructed using more than one tub of sediment, each tub should be equally apportioned among all aquaria. The sediment within each aquarium is thoroughly mixed, and remaining stones and debris are removed. If interstitial water sampling is planned, one or two Amicon^R hollow fiber interstitial water sampling units are buried 2 cm below the sediment surface. The surface of the sediment is leveled and smoothed.

Each aquarium is then filled with pond water to within 2 cm of the top. Since many types of sediment are easily resuspended, the water must be added slowly with a minimum of turbulence. A simple diffusion device, consisting of the bottom of a 4-liter polyethylene jug with holes punched around the perimeter, is useful for dissipating the force of the inflowing water. Once the aquarium is about one-half full, it is relatively easy to add more water without disturbing the sediment. If the pond water has been collected in more than one container, each container is evenly distributed to all aquaria.

If the water is murky after the aquaria are filled (cloudy enough so the bottom cannot be seen), they are allowed to settle until the water is clear. This precaution is usually unnecessary, if the water has been added carefully.

Representative samples of the macrophyte community are briefly drained (not blotted or seived, since this would damage some organisms in the community) and weighed in tared 1-liter beakers. Each microcosm

receives 100 g (drained wet wt) of the community. A few stems are pushed into the sediment to anchor the plants. After all the microcosms have been inoculated, 1 to 2 liters of the remaining water from the macrophyte collection tub are added to each microcosm as an additional source of pond organisms.

The completed microcosms are covered with clear 0.25-in. (0.64-cm) Plexiglas to retard evaporation and to protect the microcosms from dust and debris. The Plexiglas covers are also useful as supports for dissolved oxygen probes, pH probes, and other monitoring equipment. The covers must be kept clean so that light transmission is not affected.

The temperature of the environmental chamber is set to equal that of the pond. Light is supplied by an overhead bank of fluorescent tubes; cool-white tubes are satisfactory, but lights whose spectral output simulates natural sunlight are preferable. The lights are controlled by a timer on a 12-hr-on:12-hr-off cycle. Light intensity is probably not a critical variable, as long as it is constant and reproducible; in experiments performed during development of this protocol, an illumination of 16,000 lux was used. For special applications, it may be useful to simulate diurnal or seasonal fluctuations in temperature, photoperiod, or light intensity.

It is necessary to add distilled water to the microcosms periodically to replace evaporative losses. If a significant volume of microcosm water is removed in sampling, it is replaced with an equal volume of fresh pond water. No other additions to the microcosm are necessary.

Pond microcosms undergo predictable successional changes during the first two months after construction, eventually reaching a fairly

constant condition. By monitoring pH or dissolved oxygen, it is possible to detect the end of the successional period and the onset of steady-state conditions. Studies of contaminant transport, degradation, and chronic effects should be performed on equilibrated microcosms.

3.3.2 The Role of Pond Microcosms in Contaminant Research (J. M. Giddings)

Pond microcosms constructed according to the protocol (Section 3.3.1) are semi-realistic representations of shallow pond ecosystems. They differ from the natural prototypes in lacking inputs from adjacent terrestrial ecosystems, seasonal variations in light and temperature, large predators, and disturbances caused by storms. The first two items could be incorporated into the protocol without difficulty; their absence probably does not significantly affect the model ecosystem over periods of three to four months or less, since similar conditions prevail in natural ponds during the summer. Populations of some animals (zooplankton, snails, and oligochaetes) are strongly influenced by the presence or absence of fish. Ramifications of these population effects undoubtedly extend to lower trophic levels but may be insignificant; ecosystem metabolism and nutrient pools are not seriously affected (Section 3.2.1.5). The effects of storms on pond ecosystems include increased turbidity, turbulence, nutrient input, and flushing rate; sediment is resuspended, macrophytes are uprooted and washed away, and water temperature and chemistry are altered. Pond ecosystems, and organisms that inhabit them, have evolved in the context of such disturbances, and we do not know the ecological consequences of imposing constant conditions. We believe that for most experimental purposes the artificialities of our

pond microcosms do not significantly detract from their ability to mimic natural ponds. The data in Table 3.1 support this conclusion, but much more comparative research is needed.

One question which concerned us at the beginning of the project was the extent to which microcosm data would be representative of a range of aquatic environments. While our microcosms are good models of 20-cm deep ponds, they probably do not simulate deeper ponds or littoral zones of lakes as accurately, much less pelagic zones, rivers, etc. Since lakes and rivers are of greater ecological and economic importance than 20-cm deep ponds, why model the latter? The significance of pond microcosms lies in their degree of realism, which is greater than for most microcosms designed after other ecosystem types. Of all natural aquatic ecosystems, shallow ponds are least distorted by encapsulation under laboratory conditions. Moreover, it has been recognized since Forbes (1887) that processes occurring in ponds are to some extent typical of any ecosystem. Accurate simulation of a pond ecosystem is more valuable for many purposes than poor simulation of a more "important" aquatic system.

It is often supposed that an advantage of microcosms over field experiments is their simplicity, which aids in analyzing ecosystem processes. This supposition is unjustified. Whittaker (1961) noted that "aquarium communities are small but not simple; they are scarcely simpler to study and interpret than full-scale natural communities." Model ecosystems, including our pond microcosms, are too complex for analysis of individual processes, pathways, or rates. For example, it is impossible to distinguish between bioaccumulation of contaminants

through the food chain and direct absorption from water. We measure concentrations over time and calculate net rates, but we cannot isolate the various processes involved. Net rates are useful for comparisons between contaminants. The five contaminants we tested, for example, differed in the kinetic constants for disappearance from water (Table 3.14) and in bioaccumulation ratios (Table 3.30). It must be recognized that the absolute values of these parameters are not properties of the contaminants themselves but are "performances" of the system (Warren and Liss 1977). The absolute values are system-specific and cannot be applied with confidence to different aquatic environments.

Because of the difficulty of untangling components and processes in a pond microcosm, additional information about the autecology of the dominant organisms or rate constants of major processes is valuable in interpreting the results. This information may be obtained in simpler systems (the anthracene photolysis experiment conducted in conjunction with Experiment 9 is an example). In some situations, the necessary supplementary information may be available from earlier stages of contaminant testing.

A complementary approach to the use of microcosms is to measure individual processes in simple experimental systems and to integrate the results by means of a mechanistic ecosystem model. Some modelers have proposed that the only appropriate use of microcosms is the validation of models. Since microcosms can only provide estimates of net processes, validation of a model must be based on comparisons of model-derived and microcosm-derived net rates. If discrepancies are encountered, they may be due to one or more of the following factors:

Table 3.30. Summary of bioaccumulation ratios

Contaminant	Algae	<u>Elodea</u>	<u>Physa</u>	Zooplankton
As	969-2310	-	164-469	626
Cr	458-653	-	33-198	-
Se	904-1278	215-401	326	-
			726 ^a	
Hg	18,700	7400-10,600	5900-10,400	5200
			28,000-75,000 ^a	
Anthracene	712-977	816-918	953-1862 ^a	392-875

^aSnail tissue only.

- (a) inadequacies and omissions in the model;
- (b) inaccurate measurement of single processes in the simple systems;
- (c) inaccurate measurement of net rates in the microcosms;
- (d) inadequate adjustment of single process results to correct for differences between the standard test conditions and the set of environmental conditions present in the microcosms; and
- (e) interactions between individual processes or components, modifying behavior of the contaminant in unforeseen ways.

When mechanistic models do not agree with microcosm results because of factors (d) or (e), microcosm experiments provide the most direct means of predicting contaminant behavior in specific ecosystems. Microcosms simulate a natural set of environmental conditions, including their temporal and micro-spatial variations. Likewise, microcosms automatically integrate individual mechanisms, including interactions of which the modeler may not be aware. Microcosm results can thus be used to identify weaknesses in analytical models, and can provide an overall picture of contaminant behavior (under one set of quasi-natural conditions) when models fail. Models, on the other hand, can be used in conjunction with data from simplified experimental systems to assist the interpretation of microcosm results.

3.3.2.1 Transport

The contaminants tested in our transport experiments ranged from elements with relatively high natural background levels (Cr, As) to compounds not normally encountered except in polluted ecosystems (anthracene and its derivatives). In the experiments in which radio-

isotopes of arsenic, selenium, and mercury were tracked through the model ecosystem, some of the transport we measured may have represented one-half of a dynamic equilibrium between labeled and, as yet, unlabeled pools. For example, when adsorption and desorption from the sediment are equal, so that there is no net transport in either direction, we will still see an apparent movement into the sediment if we add a radioisotope to the water. In the chromium experiment (the only one in which radioisotopes were not used), we established microcosms with four levels of total chromium and measured the concentrations in various components at each level; however, the processes and pathways by which each distributional pattern was achieved can only be guessed at. Inferring cycles of naturally occurring substances is somewhat more complicated than following the dispersal of a foreign substance after it is added to the water. Most of the contaminants to undergo testing with microcosms will presumably be substances not naturally present in aquatic ecosystems. The experiment with anthracene is therefore the best example of how our microcosms might be used in contaminant transport studies.

The use of radiolabeled contaminants confers several advantages in microcosm transport experiments. Smaller samples are normally required for radioassay than for chemical analysis. Chemically undetectable contaminant levels can be monitored with radioisotopes. In theory, and often in practice, the use of labeled contaminants allows for the construction of mass budgets for the added material. Perhaps most importantly, isotopic labels are almost essential for determining the nature and fate of derivatives of the test contaminant.

Changes in the distribution of an added contaminant are rapid at first, becoming more gradual as equilibrium is approached. Accordingly,

sampling frequency must be highest at the beginning of an experiment--at least daily. We have found that water samples taken immediately after a contaminant is added do not always contain the calculated concentrations, due to incomplete mixing. Sampling the entire water column with a glass tube, rather than taking samples from a single depth, reduces this discrepancy. In most of our experiments, the contaminants became uniformly distributed in the water within one or two days.

Sediment sampling has been a major problem in our transport experiments. The oozy clay substrates we have used do not form compact cores, and a variable amount of sediment is always lost from the bottom of a core. Eloдея roots become dense as a microcosm ages, further adding to the difficulty of coring. Because of the heterogeneity of the sediment, several cores are needed for a reliable estimate of contaminant concentrations at any point in time. Ten or fifteen coring attempts are sometimes necessary to get enough good cores for each sample. The effects of repeated sampling on one microcosm could be significant. We have compromised by limiting sediment samples to the upper 1 cm, which normally contains most of the contaminant (Figs. 3.21, 3.23, Tables 3.24, 3.25). Samples of the upper centimeter are relatively easy to take (we use a glass tube connected to a syringe), and do not disturb the rest of the system appreciably. At the conclusion of an experiment, entire cores may be taken after the water has been siphoned off, and the vertical distribution of the contaminant may be accurately determined at that time.

By sampling the water, sediment, and biota at intervals throughout the experiment, we can measure (a) the distribution of the contaminant

among microcosm components, and (b) uptake of the contaminant by particular organisms. In the first case, we are interested in accounting for 100% of the contaminant over time, whereas in the second we wish to obtain precise measurements of contaminant concentrations in tissues. With all of the substances we tested, the water and sediment components together contained 90% or more of the contaminant most of the time. Water concentrations are measured more easily and accurately than sediment concentrations, so it is possible to obtain an approximate indication of the overall distribution of a contaminant by monitoring the water (Figs. 3.14, 3.19, 3.20, 3.22, 3.30, 3.38). Changes in water concentrations over time approximated exponential decay curves in nearly all of our experiments, enabling us to compare test contaminants by their first-order kinetic constants (Table 3.14). The kinetic constant reflects losses from water to organisms as well as to sediment, but generally the biota have a small effect on water concentrations. Another point of comparison for contaminant distributions is the ratio of water concentration to sediment concentration (or total contaminant mass in water to total mass in sediment). The dynamic parameter r is more convenient to estimate than the static ratios because only water samples are needed. Also, the ratios are meaningful only when the contaminant distribution has stabilized, which it sometimes fails to do. We recommend the use of r to characterize the water-to-sediment transport of test contaminants in pond microcosms.

Concentrations of the test contaminant in Elodea, algae, and Physo can be monitored throughout an experiment, but most other organisms are not abundant enough for more than a single sample. Net uptake rates

can be calculated if samples are taken frequently enough. Algae take up many contaminants very rapidly (Fig. 3.26), and require sampling every few hours for estimation of uptake rates. We have found it necessary to divide Elodea into tips (2.5 cm), stems, and roots, and to separate snails into tissue and shell, since contaminant distributions are frequently nonhomogeneous in these organisms. Elodea uptake is difficult to interpret, because uptake varies in different parts of the plant and translocation occurs within the plant as well.

Bioaccumulation ratios (concentration in organism/concentration in water) may be useful for comparisons between contaminants. Data from our experiments (Table 3.30) indicate that mercury was concentrated by all organisms to a greater extent than the other substances tested, which is in accordance with the known behavior of this element. Most bioaccumulation ratios for the other contaminants fell between 100 and 1000. There were no consistent differences between organisms. It should be pointed out that bioaccumulation ratios tend to increase over time in microcosm experiments; concentrations in organisms reach constant levels in spite of gradually declining water concentrations. The meaning of nonequilibrium bioaccumulation ratios is unclear. No significance can be attached to small differences between bioaccumulation ratios, but extreme values appear to be reliable indicators of highly bioaccumulative substances such as mercury (Table 3.30) and DDT (Metcalf 1977).

3.3.2.2 Fate

Our remarks in Section 3.3.2 concerning the difficulty of analyzing single processes in microcosms apply to fate studies as well as

transport experiments. No matter how thoroughly the microcosm components are sampled, it is difficult or impossible to reconstruct pathways of contaminant degradation from microcosm data alone. If a degradation product is detected in the tissues of an organism, it may be a product of that organism's metabolism, or it may merely have been absorbed from the water. The case of anthracene is a good example. Lu et al. (1978) tested anthracene in a Metcalf microcosm and found several derivatives in water and organisms after 33 days. They inferred biodegradation pathways within each organism from the derivatives detected in that organism. Our anthracene experiment revealed that all of the nonpolar derivatives in the microcosm biota were probably photolysis products absorbed from the water. The notion of determining degradation pathways in several species simultaneously in one experimental unit is intriguing but impractical. Microcosm data tell us about the overall fate of a compound in a whole system, integrating (and thereby obscuring) individual degradation processes. Single-species experiments, on the other hand, facilitate the analysis of individual processes, but provide no direct means of assessing overall contaminant fate. These two complementary experimental approaches are most fruitful when used in combination.

Within the context of the measurement of net degradation rates, the major limitation of a microcosm experiment is the chemical analysis of samples. Separation and quantification of organic contaminants is often difficult in the presence of complex mixtures of naturally occurring compounds. Using ^3H - or ^{14}C -labeled forms of a contaminant to be tested greatly enhances the range of information that can be derived

from a pond microcosm. The methods used in Experiment 8 (extraction of the parent compound and its derivatives in organic solvents, separation by thin-layer chromatography, location of radiolabeled spots by radioautography, and final quantification by liquid scintillation counting) could be applied to any compound available in radiolabeled form. With this procedure, information can be obtained on the net degradation rate of the parent compound in the system as a whole, the identity and quantity of derivatives of the parent compound, and the persistence of derivatives. Major degradation processes may sometimes be inferred from the data, but confirmation must come from experiments with simpler systems in which the hypothesized pathways are isolated from competing processes.

We have mentioned earlier that the sediment microbial community is probably the most realistic component of pond microcosms, although this assumption needs to be verified by a competent aquatic microbial ecologist. The rest of the detrital food web is also well represented; protozoa, nematodes, rotifers, gastrotrichs, and oligochaetes are abundant and diverse. The decomposer community appears to be little affected by enclosure in an aquarium or by the exclusion of large predators. Since most of the biodegradation of organic contaminants in aquatic ecosystems occurs in the sediment microbial community, the fate of a test compound in microcosm sediments is a reliable indication of its fate in nature. Such data are quite valuable because even qualitative information on the persistence of a compound and its derivatives is difficult to obtain experimentally (this was the subject of a recent workshop sponsored by the Gulf Breeze Environmental Research Laboratory of the EPA).

4.0 TERRESTRIAL STUDIES

4.1 INTRODUCTION

4.1.1 State of the Art, Criteria, and Rationale

The purpose of this portion of the project was to determine the value of microcosms as test systems to examine the rates of transport, transformation, accumulation, and effects of chemical contaminants in terrestrial environments. A recent review of microcosm construction and manipulation suggested six critical study areas (Witherspoon et al. 1976). First, the effects of biotic community complexity on rates and pathways of chemical transport required determination. Effects of the decomposer community on elemental transport and accumulation are especially great. The presence of primary producers, whether grasses or trees, significantly alters transport rates and chemical states of contaminants. Second, information about the replicability and reproducibility of microcosms was needed. A major shortcoming of microcosm studies conducted to date has been the uniqueness of experiments; very few have been repeated and the results compared. Third, the determination of the optimal size of a microcosm for realistic estimation of contaminant fate was necessary for managed (pastures) and natural (forests) ecosystems potentially receiving contaminant inputs. Two major ecosystem processes affecting chemical transport and fate, primary production, and decomposition, have successfully been studied, using small experimental units in separate microcosm and pot studies. Microcosms constructed to document the transport and fate of contaminants should incorporate these experimental units to ensure the realistic

functioning of their environmental components. Therefore, relative size becomes an important determinant, in both forest and pasture analogs, for reliable estimation of conditions in the field. Fourth, determinations of equilibration time necessary for newly constructed microcosms and identifiable and measurable parameters to monitor within microcosms during equilibration and experimentation were required. Equilibrium, for the purposes of these microcosm studies, is defined as that state from which reproducible metabolic parameters (such as CO_2 efflux) can be expected from field-derived soil cores or larger soil sections collected at different times. Fifth, the response time of each microcosm component to contaminant additions (dose, incubation time, chemical form) until equilibration with the chemical is reached was needed. Chemical redistribution and transformation within microcosms was assessed after disturbance to or removal of critically important microcosm components. Finally, comparisons were needed between rate parameters and transformation products determined in microcosms and those occurring in the field. Field values and ecosystem models were available from the IBP and other ecosystem analysis studies. These comparisons allowed preliminary extrapolation of microcosm results to the field; field validation was not within the scope of this study.

4.1.2 Summary of Research Strategy

The approach taken in these studies was to assess the importance of size, complexity (abiotic and biotic), replicability, and stability of microcosms in each of two ecosystem types, a pasture and a forest. The construction of microcosms and the analysis of these as test systems began in the first year. Size, replicability, and biotic complexity of

microcosms from pasture and forest were assessed using routine measurements of certain ecosystem processes (CO_2 efflux, plant growth, consumers, population growth, etc.). These routine measures provide a basis from which to establish equilibration times for the microcosms and stability after equilibrium. In addition, comparisons were made between values obtained from these metabolic measurements and those from other microcosm and field studies to assess microcosm reality.

Emory silt loam soil was chosen as the forest soil because of knowledge gained in previous experiments. This soil is a bottom-land soil, alluvial in origin, and found in many study areas on the ORNL reservation (Table 4.1). The soil is slightly acid (pH 5.5) and moderately high in organic matter (4.7%). The soil has been studied in detail and its physicochemical characteristics documented.

Pasture soil used in microcosms was a Captina silt loam from a grassland study area on the ORNL reservation. This is a well-drained, fairly fertile (3.2% organic matter), slightly acid (5.6 pH) soil occurring near the Clinch River (Table 4.1).

The two terrestrial ecosystems were chosen as those for which we have complementary field and microcosm data. The suite of plants and microbial and invertebrate populations used in microcosms will reflect the ecosystem types chosen. The three microcosm sizes were chosen for (1) simplicity, ease of handling, and replicability (2.5 x 5 cm); (2) use in other studies (5 x 15 cm); and (3) suitability for tree seedling growth (46 x 46 x 15 cm) and grass growth (46 x 46 x 15 and 5 x 15 cm). Within the grass and forest microcosms, biotic complexity was defined to include various combinations of biota known to modify

Table 4.1. Chemical and physical characteristics of experimental soils

Ecosystem	Soil	pH	CEC (meq/100 g)	Organic matter (%)	Clay (%)	Silt (%)	Sand (%)
Forest	Emory silt loam	5.5	10.6	4.7	14.8	58.6	13.6
Grassland	Captina silt loam	5.6	8.5	3.2	63.7	29.1	7.2

some chemical transfer rates and transformations (i.e., presence or absence of microbes, invertebrates, and plants).

4.2 SUMMARY OF RESULTS (W. F. Harris, ed., from contributions by B. S. Ausmus, D. R. Jackson, E. G. O'Neill and P. Van Voris)

Evaluation of terrestrial microcosms was initiated in recognition of their potential application as screening tools for assessment of contaminant transport. To this end, first-year activities included evaluation of microcosm construction, complexity, stability, and reproducibility (Van Hook et al. 1976). Emphasis solely on contaminant transport was broadened to include investigation of contaminant effects that might occur, based on early identification of sensitive system-level monitoring points in microcosms. Thus, second-year activities were undertaken to: (1) further examine and document the validity of nutrient export and CO_2 efflux as sensitive measures of ecosystem effects, (2) continue examination of chemical transport in microcosms, and (3) refine and document protocols for standardizing microcosms for use as screening tools for chemical contaminants. The third-year effort on terrestrial microcosms involved no new experimental work. The extensive data sets developed in the first and second years were subjected to further analysis across experiments to elucidate patterns of terrestrial microcosm behavior. A second major analytical task in the third year dealt with application of simulation modeling to microcosm and field data as a means of (1) extending the interpretation of microcosm results in time, and (2) through the use of sensitivity analysis, establishing the range of physical and biotic conditions in the terrestrial environment that need to be included in measurements on terrestrial microcosms.

4.2.1 Behavior of Heavy Metals in Forest Microcosms - Excised Tree Microcosms

This experiment (TREETECOSM I) was conducted to assess the transport, fate, and effects of heavy metals on forest microcosms. Specifically, objectives were to: (1) document the transport and assimilation of Pb, Cd, Zn, and Cu in forest components, and compare microcosm results to similar findings reported on Crooked Creek Watershed (CCW) (Jackson and Watson 1977); (2) document the effects of heavy metals on nutrient cycling within forest microcosms, and verify that the observed changes in nutrient dynamics of CCW were indeed the result of heavy metal contamination; and (3) discern the effect of heavy metal contamination on litter-soil biota in forest microcosms. Efflux of CO_2 was used as an index of biotic activity because it can be monitored nondestructively and has been historically viewed as a measure of decomposer community respiration (Gray and Williams 1971, Parkinson et al. 1971). In this study CO_2 efflux was used as an indicator of the integrated litter-soil community dynamics, including roots.

4.2.1.1 Materials and Methods

Six intact microcosms (45 x 45 x 25 cm) were excised from a mesic hardwood forest near Oak Ridge, Tennessee (see Ausmus et al. 1976 for site description) in December 1974. Soil was alluvial, Emory silt loam (% clay = 28, % silt = 59, % sand = 13), slightly acidic (pH = 5.5), with approximately 5% organic matter. Each soil section contained an Acer rubrum sapling approximately 2 m in height and the associated ground flora. Sections were placed in wooden boxes, set against two

walls and sealed with quick-setting epoxy resin. Microcosms were placed in a greenhouse and monitored for a period of 20 months.

Microcosms were equilibrated for two months, December and January, then litter layers were removed and weighed. Litter from control microcosms was replaced after weighing. Original litter from three microcosms designated for heavy metal treatment was replaced with equivalent weights (35 g O1 litter, 100 g O2 litter) of litter contaminated with heavy metals collected 0.4 km from the smelter on Crooked Creek Watershed (CCW) (Watson et al. 1976). After three weeks, additional heavy metal input to treated microcosms was added in the form of baghouse dust obtained from the smelter adjacent to CCW. Dust was applied weekly as an aqueous slurry for 12 weeks, approximating one annual deposition which would occur 0.4 km from the smelter stack on CCW (Jackson and Watson 1977). The total combined dose to the treated microcosms was: 1 mg/cm^2 Pb, 0.13 mg/cm^2 Cd, 0.75 mg/cm^2 Zn, and 0.16 mg/cm^2 Cu (Table 4.2). Lead and Cu were primarily associated with contaminated litter, while the primary source of Cd and Zn was baghouse dust (Table 4.2). Soil leachate was collected biweekly following addition of distilled water in excess of field capacity and analyzed for heavy metals (Pb, Zn, Cu, and Cd) by flame atomic absorption and for nutrients (Ca, Mg, and K, and $\text{NO}_3\text{-N}$ and PO_4 by flame atomic absorption and Autotechnicon techniques, respectively).

Blackened plastic boxes approximately one-fourth the surface area of a microcosm were inverted over alkali traps (25 ml, 0.18 M KOH) for 24-hr periods. Carbon dioxide efflux was calculated from titration data using 0.10 M HCl as acid titrant. Open and closed blanks were

Table 4.2. Source of heavy metals (Pb, Zn, Cd, Cu) deposited as contaminated litter and as baghouse dust on forest microcosms

Metal source	Pb		Cd		Zn		Cu	
	(mg/cm ²)	(% dose)	(mg/cm ²)	(% dose)	(mg/cm ²)	(% dose)	(mg/cm ²)	(% dose)
01 litter	0.6	5.4	0.003	2.3	0.021	2.8	0.007	4.3
02 litter	7.9	71.8	0.015	11.7	0.217	29.0	0.148	91.9
Baghouse dust ^a	2.5	22.7	0.110	85.9	0.510	68.2	0.006	3.7
Total dose	11.0	100	0.128	100	0.748	100	0.161	100

^aTotal of 12 weekly applications.

used to subtract ambient CO_2 concentrations from CO_2 efflux estimates. Between yeardays 300 and 350, CO_2 traps were not maintained, resulting in a gap in the data base. Other parameters monitored were: ambient air temperature, soil moisture, nutrient concentrations in soil leachate, leaf nutrient concentrations, and aboveground plant growth.

Six Acer leaves were collected monthly during the first growing season and analyzed for heavy metal content. Foliage from each microcosm was pooled and ashed at 450°C for 24 hr. Ash was dissolved in 25 ml 0.1 N HCl and analyzed for Pb, Cd, Zn, and Cu.

Microcosms were harvested during mid-September 1976. Microcosm components were separated, dried, ashed, and dissolved in 0.1 N HCl prior to heavy metal analysis. Root tissue (< 0.5 cm diam., > 0.5 cm diam.) was thoroughly washed to remove adhered soil particles. Soil was partitioned into three depths, 0-5, 5-10, and 10-25 cm, and heavy metals were extracted by shaking 5-g aliquots of dried and sieved soil with 25 ml 0.1 N HCl.

A new procedure for the determination of extractable nutrient pools (Jackson and Hall 1978) was used at harvest to extract soil (0-5 and 5-10 cm depths) from treated and control microcosms (see Section 4.2.6 for technique description). The technique involved excision of cores (5 cm diam. x 10 cm depth) from microcosms, division of cores into 5-cm depths, and sealing in heat shrinkable polyvinyl chloride. Aliquots of 200 ml each 1 M NaHCO_3 were leached through each core. The resulting KCl and NaHCO_3 extracts were analyzed for Ca and $\text{NH}_3\text{-N}$; and $\text{PO}_4\text{-P}$, $\text{NO}_3\text{-N}$, and dissolved organic carbon (DOC), respectively.

Soil microbial communities were characterized using three parameters. Total soil microbial protoplasm was estimated using the ATP assay by extraction of 1 g soil with 3 ml chloroform mixed with 6 ml of 0.02 M TRIS buffer (pH = 7.4). After centrifugation, 2 ml CCl_4 were added to the decanted buffer to remove traces of chloroform. The aqueous phase of each solution was then assayed for ATP by measuring absorbance of NADH (340 nm) produced by enzymatic (hexokinase) reaction of ATP with glucose. Bacterial density and fungal mass were determined by standard dilution plating (Parkinson et al. 1971) and modified Jones-Mollison slide technique (Jones and Mollison 1948, Parkinson et al. 1971), respectively.

4.2.1.2 Results and Discussion

Transport and Distribution Among Components

Characterization of baghouse dust. Chemical analysis of baghouse dust revealed Pb, Cd, Zn, and Cu concentrations of 590, 26, 120, and 1.5 mg/g, respectively. Analysis of the dust by x-ray diffraction indicated the presence of metal sulfates, oxides, and sulfides. In addition, metals in the dust were readily soluble in 0.01 M EDTA (Fulkerson et al. 1974). Heavy metal particulates removed from leaf surfaces on CCW were similar in chemical composition and solubility to the baghouse dust (Jackson and Watson 1977).

Transport and distribution between soil and vegetation. All metals (Pb, Cd, Zn, and Cu) in treated microcosms were significantly greater in concentration down to a soil depth of 25 cm relative to the controls (Table 4.3). Export of dissolved metals in soil leachate of treated

Table 4.3. Heavy metal concentrations in litter, soil, and vegetation (*Acer rubrum*) components of forest microcosms (N=3)

	Control (mg/g)				Treated (mg/g)			
	Pb	Cd	Zn	Cu	Pb	Cd	Zn	Cu
Litter								
01	10	0.4	84	18	7,800	68	410	190
02	80	0.5	48	26	11,000	100	760	520
Soil								
0-5 cm	24	0.1	10	8	1,100	9.0	40	20
5-10 cm	18	0.02	4	4	320	3.8	40	10
10-25 cm	19	0.02	6	6	460	3.2	18	10
Leaves	7	0.6	29	7	320	6.4	56	18
Branches	2	0.2	30	4	22	1.1	180	4
Bole	3	0.2	15	4	62	0.6	27	4
Roots								
< 0.5 cm diam	10	1.0	32	11	29	1.8	115	11
> 0.5 cm diam	11	0.2	39	5	23	0.3	88	4

microcosms, although greater than controls, was less than 0.2% of the total applied dose (Table 4.4). These results reflect the mobility of metals from baghouse dust and contaminated litter, as well as the great capacity of soil for retention of heavy metals.

The sites of greatest concentration of metals in the microcosms was 02 litter and 0-5 cm soil in both treated and control microcosms (Table 4.2). These results reflect the presence of humic substances, predominant in 02 litter and surface soil, which have high affinity for heavy metals. The 02 litter was also the site of greatest heavy metal accumulation on CCW (Watson et al. 1976).

Analysis of leaf tissue during the first growing season (February-November 1975) indicated no significant uptake of heavy metals. However, uptake of all heavy metals was significant during the second growing season, as indicated by metal concentrations in leaves at the time of harvest (Table 4.3). These results show a lag time of one growing season before significant translocation of metals occurred into Acer leaf tissues.

In most cases the distribution of metals in Acer rubrum of treated microcosms was different from the control microcosms. For example, Pb in the bole component of treated microcosms was comparable to Pb in leaves and roots; whereas, in controls the bole contained significantly lower Pb concentrations than either leaves or roots. Thus, mechanisms of heavy metal translocation within Acer rubrum were apparently different in the presence of highly concentrated metals from smelter emissions.

Enrichment in soil and vegetation. Enrichment ratios (ER) were computed by dividing the heavy metal concentration of a treated

Table 4.4. Total leachate export of heavy metals from forest microcosms over the 20-month experiment

Treatment	Pb		Cd		Zn		Cu	
	($\mu\text{g}/\text{cm}^2$)	(% dose)	($\mu\text{g}/\text{cm}^2$)	(% dose)	($\mu\text{g}/\text{cm}^2$)	(% dose)	($\mu\text{g}/\text{cm}^2$)	(% dose)
Smelter								
emissions	1.62 ^a	0.01	0.12 ^a	0.01	1.27 ^a	0.1	0.45 ^a	0.2
Control	0.62	--	0.05	--	0.13	--	0.09	--

^aSignificantly greater than control ($P \leq 0.05$).

microcosm component by the same metal concentration of a control microcosm component, i.e., for Pb in leaves, $ER = 320/7 = 45$ (Table 4.5). These ratios were computed to evaluate the mobility of heavy metals in treated microcosms relative to controls.

With the exception of Cu in branches, bole, and roots, all metals in every component of treated microcosms were enriched ($ER > 1$) (Table 4.5). Within Acer rubrum, the greatest enrichment occurred in leaf tissue for all metals except Cu. All metals were least enriched in root tissue. Enrichment ratios show that heavy metals are greatly dispersed within Acer tissue after assimilation by roots.

Greatest enrichment of metals in soil occurred in 01 and 02 litter (Table 4.5). This was primarily the result of placing highly contaminated litter on the surface of treated microcosms. Cadmium was more enriched in soil than the other metals. This result reflects the extremely low indigenous concentration of Cd in control soil (Table 4.3) as much as the high dosage to the treated microcosms. Enrichment of Zn and Cu was more uniform through the soil profile than Pb or Cd, suggesting that the mobility of Zn and Cu in contaminated soil was more similar to that in controls than that in Pb and Cu.

Effects on Nutrient Cycling Processes

To discern differences in initial litter substrate quality between treated and control microcosms which might bias experimental results, several parameters were measured. These were: total carbon, labile carbon (water- and ethanol-soluble), nitrogen, phosphorus, and calcium (Table 4.6). There were no significant differences in total carbon, labile carbon, or nitrogen between contaminated and control litter

Table 4.5. Heavy metal enrichment ratios for forest microcosms

Component	Pb	Cd	Zn	Cu
Vegetation				
Leaves	45.0	10.7	1.9	2.6
Branches	10.4	5.5	5.6	0.8
Bole	26.8	3.0	1.8	1.0
Roots (< 0.5 cm)	2.9	1.3	3.6	1.0
Roots (0.5-2.0 cm)	2.1	1.5	2.3	0.9
Soil				
01 litter	783.0	168.5	4.9	10.7
02 litter	138.0	214.0	16.0	20.0
0-5 cm	44.2	89.0	4.1	2.5
5-10 cm	18.2	190.0	10.2	2.8
10-25 cm	24.5	160.0	3.0	2.4

Table 4.6. Initial litter and soil nutrient pools in contaminated and control microcosms

Stratum	Microcosm	Total C	Labile C ^a (mg/cm ²)	N	Ca	P (μg/cm ²)
01 litter	Treated		3.7	0.14	0.12	6.5
	Control	8.1	4.0	0.18	0.43 ^b	17.3 ^b
02 litter	Treated		6.9	0.94	0.46	48.3
	Control	34.7	7.3	1.26	4.35 ^b	167.9 ^b

^aLabile C = water- and ethanol-soluble carbon.

^bSignificantly different at $P \leq 0.05$.

($P \leq 0.05$). Initial calcium and phosphorus concentrations in O1 and O2 litter of control microcosms were greater than those in treated microcosms (Table 4.6). Therefore, slightly greater nutrient turnover and decay rates may be expected in control microcosm litter, but were not measured in this study.

Nutrient leachate. Total export of Ca, Mg, K, and $\text{NO}_3\text{-N}$ was significantly increased from treated microcosms during the 20-month experiment (Table 4.7). Export of $\text{PO}_4\text{-P}$ was not affected by heavy metal contamination. The variance of nutrient leachate among replicate microcosms was also increased in treated microcosms.

Greatest losses of nutrients from treated microcosms were Ca and $\text{NO}_3\text{-N}$. Similar results have been reported in response to other stresses, e.g., arsenic (Jackson et al., 1978) and clear-cutting (Likens et al. 1970). However, significant $\text{PO}_4\text{-P}$ did not occur in response to heavy metal contamination. Similarly, P was not greatly depressed near the smelter at CCW (Jackson and Watson 1977).

Nutrients in litter and *Acer rubrum*. Over the 20-month experiment, litter mass did not significantly change in either treated or control microcosms (Table 4.8). While litter nutrients were lost from control microcosms during the period, contaminated litter retained or gained nutrients (Table 4.8). This is consistent with observations of increased nutrient retention in response to heavy metal contamination at CCW (Jackson and Watson 1977). However, these results cannot definitively validate field results because of the initial differences between contaminated and control litter substrate quality.

Table 4.7. Total dissolved nutrient export from contaminated and control forest microcosms over the 20-month experiment^a

Microcosm	mg total export/microcosm				
	Ca	Mg	K	PO ₄ -P	NO ₃ -N
Treated	216	59.5	39.0	133	42
	(47)	(11.6)	(5.1)	(30)	(36)
Control	169	46.5	26.7	158	6.5
	(1.5)	(1.3)	(3.4)	(12)	(5.9)

^aNumbers in parentheses are standard errors.

Table 4.8. Final litter nutrient pools and total changes in nutrient pools over the 20-month experiment

Stratum	Microcosm	(mg/cm ²)							
		Mass	N	P	Ca				
01 litter	Treated	15.6	1.7	0.15	+0.01	7.65	-1.15	0.32	+0.2
	Control	17.0	0.3	0.16	-0.2	8.89	-8.41	0.27	-0.16
02 litter	Treated	106.6	7.8	1.04	+0.10	65.9	+17.6	1.38	+1.08
	Control	92.7	-6.1	0.71	-0.55	33.0	-13.5	1.24	-3.11

Distribution of nutrients in Acer rubrum tissue did not significantly differ between contaminated and control microcosms (Table 4.9). Leaf and root tissue sampled on CCW near the smelter had depressed nutrient concentrations relative to those sampled in control areas (Jackson and Watson 1977). Presumably, primary producer exposure to heavy metal concentrations requires more than two growing seasons to cause depressed nutrient uptake due to the great reservoir of nutrients solely within the tree. Depressed nutrient assimilation by trees could result from depleted nutrient pools in soil, damaged mycorrhizal associations, or both. As shown in Fig. 4.1, extractable soil nutrients were depleted by heavy metal contamination due to increased leaching. While mycorrhizal structures were not assessed in this experiment, chemical toxins have been reported to affect mycobionts (Trappe et al. 1973).

Effects on Litter-Soil Carbon Metabolism

Carbon dioxide efflux from control and contaminated microcosms was weakly correlated with temperature ($r \approx 0.6$). Neither soil moisture nor watering schedule was significantly correlated with daily CO_2 efflux.

Rates of CO_2 efflux. Contamination of litter-soil strata with heavy metals significantly increased daily CO_2 efflux rates following initial treatment ($P \leq 0.05$). To closely examine CO_2 loss, two periods of each year were partitioned: spring (mid-February, year-day 50, to early May year-day 124) and summer (early May, year-day 125, to mid-July, year-day 200). This partition was made to compare CO_2

Table 4.9. Mean nutrient concentrations of forest microcosm components at experiment termination

Component	Microcosms	(mg/g dry wt)				
		Ca	Mg	K	P	N
Leaves	Treated	6.60	1.45	4.25	0.78	14.8
	Control	6.10	1.55	5.70	1.26	13.1
Branches	Treated	5.40	0.48	1.43	0.38	5.9
	Control	7.70	0.64	1.18	0.42	3.4
Bole	Treated	6.31	0.29	0.96	0.32	5.5
	Control	2.82	0.55	0.79	0.36	3.3
Roots (< 0.5 cm)	Treated	5.70	1.14	1.63	0.67	12.3
	Control	6.31	1.33	2.64	0.86	9.1
Roots (> 0.5 cm)	Treated	5.42	0.72	1.15	0.55	9.4
	Control	5.63	0.89	1.52	0.75	8.1
01 litter	Treated	20.9	2.67	1.49	0.49	7.5
	Control	15.6	1.74	1.89	0.52	1.0
02 litter	Treated	13.0	1.38	7.9	0.62	9.9
	Control	13.4	1.34	8.0	0.36	7.5
Ground flora	Treated	11.5	3.01	7.56	0.74	
	Control	9.9	3.08	28.32	0.82	

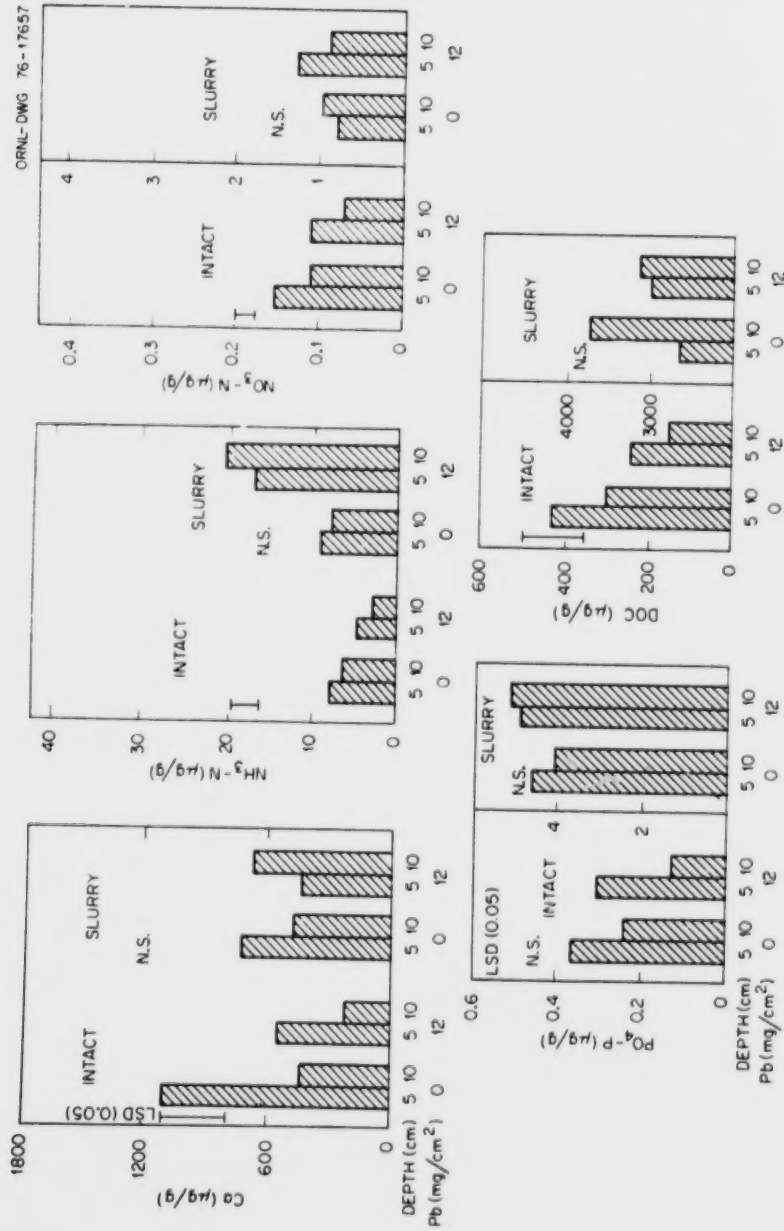


Figure 4.1. Effect of heavy-metal contamination on extractable soil nutrients by the intact and slurry methods. Vertical bar denotes least significant difference at $P < 0.05$.

efflux between periods of two consecutive years having similar temperature-moisture regimes and equal numbers of observations ($n = 75$).

During the first year, CO_2 efflux rates averaged 3.20 and 2.60 mg/day from contaminated and control microcosms, respectively (Table 4.10). Carbon dioxide efflux rates from control microcosms remained constant through spring of the second year. During summer of the second year, CO_2 efflux rates from both contaminated and control microcosms decreased to a level of 2.0 mg CO_2 /microcosm/day (Table 4.10).

Total gaseous carbon loss. Cumulative CO_2 efflux was computed by summing daily CO_2 efflux rates and expressed as a regression against time. Due to the gap in the data base, total gaseous CO_2 loss was computed separately for the first (year-days 0-300) and second years (year-days 0-205).

Total gaseous carbon loss between treated and control microcosms was identical until year-day 130, after which time greater loss was incurred from the treated microcosms. These results indicate that the application of highly contaminated litter did not significantly affect total gaseous carbon loss until three months post-treatment. After this time, however, CO_2 loss from control microcosms remained constant, while CO_2 loss from treated microcosms was increased as indicated by a greater regression coefficient between years 1 and 2 (Table 4.11).

Regression analysis (Table 4.11) indicates that cumulative CO_2 efflux from control microcosms did not differ between years. Therefore, the control data were pooled into a single regression equation for both

Table 4.10. Comparison of average CO₂ efflux rates (mg CO₂/microcosm/day) of contaminated and control microcosms during spring and summer of the two years of observations

Year	Spring (year-days 50-124)		Summer (year-days 125-200)	
	Contaminated	Control	Contaminated	Control
1	3.20 ^a	2.60	3.20 ^{a,b}	2.60 ^b
2	3.46 ^{a,c}	2.67	2.00	2.00

^aSignificantly greater than controls at $P \leq 0.05$.

^bYear 1 > year 2 at $P \leq 0.05$.

^cYear 2 < year 1 at $P \leq 0.05$.

Table 4.11. Simple linear regression of cumulative CO₂ efflux (mg) upon time (days) for contaminated and control microcosms for the two years of experimentation

Year	Treatment	N	Intercept	Slope	F	R ²
1	Contaminated	203	-14.8	0.495 ^a	10,000 ^c	0.98 ^d
	Control	203	-11.8	0.450	9,741 ^c	0.98 ^d
2	Contaminated	186	-8.9	0.635 ^{a,b}	11,596 ^c	0.98 ^d
	Control	186	-5.1	0.476	12,915 ^c	0.99 ^d
1,2	Control	389	-4.6	0.433	4,238 ^c	0.92 ^e

^aSignificantly different from controls at $P \leq 0.05$.

^bSignificantly different from year 1 at $P \leq 0.05$.

^cSignificantly linear regression at $P \leq 0.01$.

^dSignificantly fit with a + bx model at $P \leq 0.01$.

^eSignificantly fit with a + bx model at $P \leq 0.05$.

years of the experiment. Efflux rates of CO_2 were independent of soil moisture and weakly correlated with soil temperature ($r = 0.60$). Thus, variable environmental conditions in the greenhouse did not significantly affect CO_2 efflux during the experiment.

Microbial community characterization. The three parameters measured to characterize the soil microbial community were significantly different between contaminated and control microcosms at soil depths of 0 to 5 and 5 to 10 cm. Results indicate that metal contamination increased ATP concentration and bacterial density and decreased fungal biomass (Table 4.2). Elevated ATP concentrations resulted from the elevated bacterial biomass, which more than compensated for decreased fungal biomass in soil of contaminated microcosms.

4.2.2 The Behavior of Arsenic in Terrestrial Microcosms

The purpose of this research was to evaluate the use of soil microcosms for screening chemicals potentially toxic to terrestrial ecosystems, and to determine the sensitivity of monitoring Ca and $\text{NO}_3\text{-N}$ efflux from forest and grassland soil treated with subacute levels of arsenic.

Mesic hardwood forest and managed pasture were chosen for study because they are dominant ecosystems of the southeastern United States and represent possible targets for chemical contaminants in this region. In addition, these ecosystems are represented on the Oak Ridge Reservation and have been monitored extensively. Their selection allows comparison of parameters measured on microcosms to field-established values. Soil characteristics of these sites have been summarized in Table 4.12.

Table 4.12. Microbial parameters in soil of contaminated and control microcosms at the end of the 20-month experiment

Microbial parameter	Treatment	Soil	
		0-5 cm	5-10 cm
ATP concentration (ppm)	Contaminated	13.7 ^a	4.2 ^b
	Control	5.6	2.1
Bacterial density (10 ⁸ /g)	Contaminated	21.8 ^a	10.2 ^b
	Control	8.0	5.1
Fungal lengths (m/g soil)	Contaminated	75 ^a	135 ^a
	Control	1300	750

^aSignificantly different from controls at $P \leq 0.01$.

^bSignificantly different from controls at $P \leq 0.05$.

The mesic hardwood study area is within a 30-ha (70-acre), second-growth mesophytic assemblage dominated by Liriodendron tulipifera L. Mean canopy height is 30 m; mean stand age is 48 years. The climatic regime is humid-mesothermal with a mean annual soil temperature of 13°C. This system is characteristic of southern Appalachian Highland cove hardwoods in karst topography and has been the intensive study site in the Eastern Deciduous Forest Biome of the International Biological Program.

The managed pasture study area is comprised of 16 ha (40 acres) and is characterized by codominance of Festuca arundinacea Schub. and Andropogon virginicus L. The pasture has a perched water table during winter which causes saturated flow in the silt loam soils during this high rainfall period. The pasture was in agricultural use prior to 1942 and planted in Pinus taeda L. in 1956 after lying fallow for 14 years. In 1964, the pines were cut and the area planted in Festuca. Mean annual temperatures resemble the forest but extremes are greater. The pasture has received occasional mowing to maintain Festuca dominance.

4.2.2.1 Materials and Methods

Nine soil cores (5 cm diam. x 10 cm depth) each were excised from a managed grassland and a mesic forest. Growth of plants was eliminated from each grassland microcosm by cutting stems at the soil surface. All soil cores were encased in shrinkable PVC plastic sheeting and placed on a filter holder to collect leachate. The cores were incubated in a temperature-regulated growth chamber at 25°C. Leachate was

collected weekly by adding enough water (20-30 ml) to exceed field moisture capacity and supply approximately 10 ml in the leachate collector.

Microcosms were treated with As solutions containing ^{74}As tracer. Solutions contained specific activities of 1.09×10^{10} and 1.08×10^9 dpm $^{74}\text{As/g As}$ for application rates of 10 and 100 $\mu\text{g As/cm}^2$, respectively. Three microcosms were assigned to each treatment level. Each treatment was applied by leaching As solutions through individual microcosms. Retention of As by soil was 40 to 60%; therefore, final As concentrations in soil cores were more indicative of actual As treatments than the initial application rate (Table 4.13).

Leachate solutions were filtered through Whatman 40 paper and analyzed for pH. Subsamples of 5 ml each were placed in counting tubes for radiochemical determination of ^{74}As . Samples originating from untreated soil cores were not routinely assayed for As. However, analysis of two leachate samples from an untreated core indicated an As concentration at least 1000-fold lower than leachates from As-treated soil cores. Therefore, background As in these soils was assumed not to be a factor in the experiment. Analytical determinations for Ca were made by flame atomic absorption, and Autotechnicon procedures were used for determination of $\text{NO}_3\text{-N}$.

4.2.2.2 Results and Discussion

Transport and Effects of Arsenic

The depth-profile of As concentrations in forest and grassland microcosms indicates greatest retention in the top 2.5 cm of soil

Table 4.13. Final As concentrations of As-amended forest and grassland soil cores ($\pm$ S.E., n=3)

Soil type	Soil depth (cm)	As treatment	
		10 ($\mu\text{g}/\text{cm}$)	100
Forest	0-2.5	5.2(1.1)	34.9(11.2)
	2.5-5.0	1.0(0.5)	7.3(1.6)
	5.0-10.0	1.0(0.3)	8.0(1.8)
Grassland	0-2.5	4.7(1.4)	24.6(2.6)
	2.5-5.0	1.1(0.2)	4.3(1.5)
	5.0-10.0	0.5(0.1)	3.8(0.2)

(Table 4.13). Presumably, As was retained in this area by humic substances. Forest soil retained a greater concentration of As than grassland, which may reflect a slightly higher organic matter content of the forest soil (Table 4.1). The highest level of As retention (34.9 ppm) in this experiment is well below a value of 90 ppm reported to cause reductions in plant growth (Carrow et al. 1975). Therefore, the levels of As used in this study were considered subacute for terrestrial ecosystems.

Efflux of Ca and $\text{NO}_3\text{-N}$ was significantly increased ($P \leq 0.05$) from forest microcosms amended with the highest concentration of As (Table 4.14). Grassland microcosms were unaffected at this level of amendment. Forest microcosms appear more sensitive to contamination by As. This effect may be due to the greater retention of As by forest soil. Various indicators of microbial activity (amylase, cellulase, invertase, and urease enzyme activity and ATP concentration) failed to detect a treatment effect for both soils and levels of As amendment.

Nutrient Comparisons

Calcium efflux diminished for approximately four weeks from untreated microcosms of both ecosystems (Fig. 4.2). Similar results were obtained from treated microcosms which were unaffected by As amendment. Physical disruption of the soil system created by the excision process may account for the high initial concentrations of Ca. A pre-equilibration period of approximately four weeks is recommended for future experiments on the basis of this study.

Table 4.14. Aqueous efflux of Ca and NO₃-N from forest and grassland soil cores amended with As ($\pm$ S.E., n=18)

Soil type	As treatment ($\mu\text{g}/\text{cm}^2$)	Ca efflux ($\mu\text{g}/\text{ml}$)	NO ₃ -N efflux ($\mu\text{g}/\text{liter}$)
Forest	0	20.3(1.7)	32.4(4.8)
	10	24.7(3.8)	48.9(7.5)
	100	29.4(2.9)	132.2(26.0)
Grassland	0	10.6(1.3)	20.8(3.5)
	10	12.5(2.2)	23.3(5.0)
	100	11.3(0.9)	39.9(24.0)

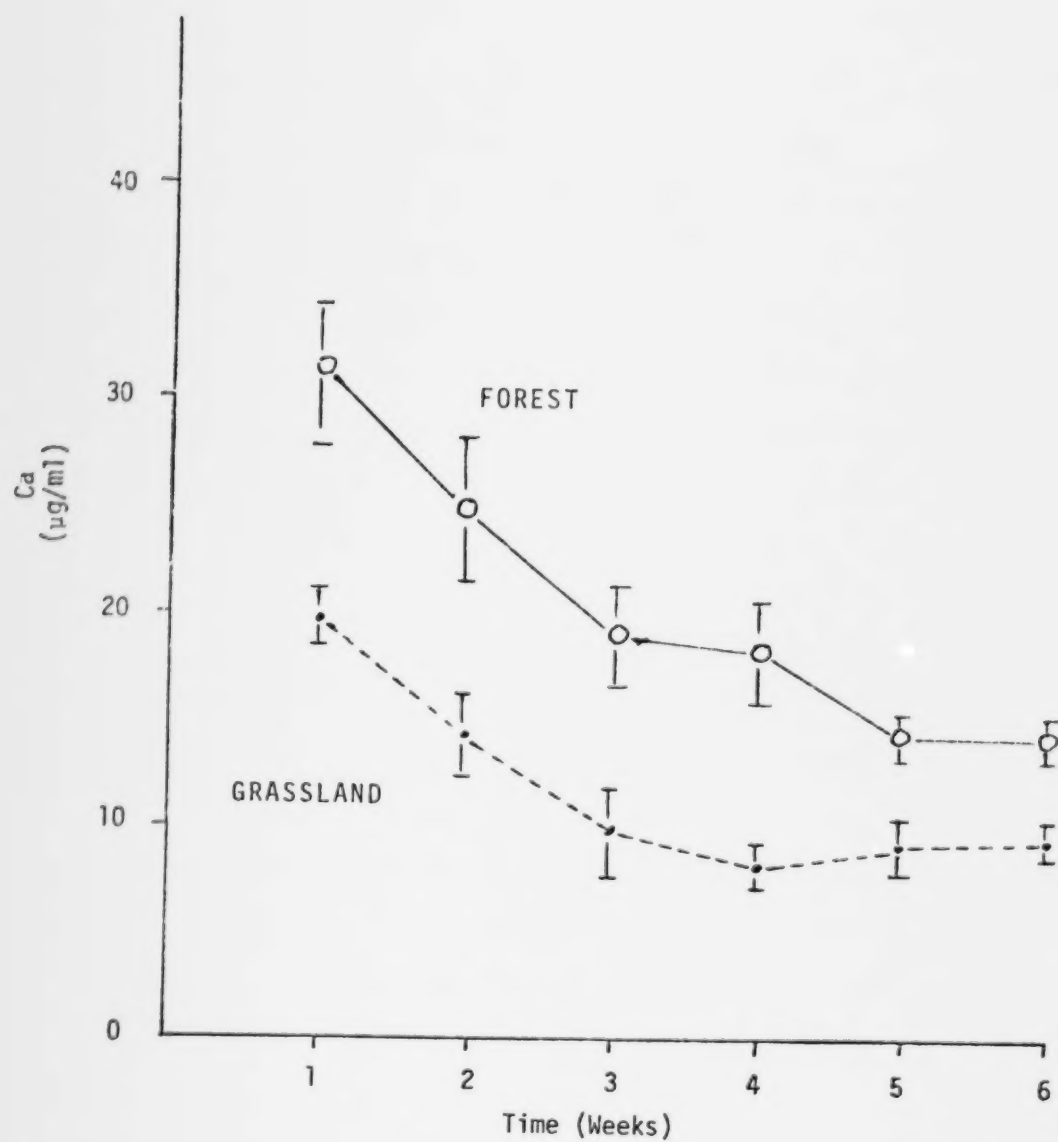


Figure 4.2. Mean weekly Ca efflux from untreated forest and grassland soil cores.

4.2.2.3 Summary and Conclusions

This study supports the use of intact soil cores for screening chemicals which may be toxic to terrestrial ecosystems. Nutrient efflux from the cores was stabilized within four weeks, and the cores were amenable to routine handling. Treatment of the soil cores with a sub-acute dose of As resulted in increased nutrient efflux from forest soil, while population parameters were unaffected. These results suggest that efflux of nutrients in soil water is a more sensitive monitoring point than population parameters for assessing chemical stress in soils. Nutrient losses appear to provide an early warning indicator of more drastic effects from the exposure to toxic contaminants.

4.2.3 Extraction of Nutrients from Intact Soil Cores

A sensitive soil extraction technique could be invaluable for assessing nutrient pools in soil cores at the end of a prescribed incubation period. This technique might be more quantitative (in terms of assessing changes in nutrient pools) and more economical than monitoring nutrient concentrations in soil leachate.

The technique developed to extract nutrient pools from intact soil must be: (1) highly sensitive to slight changes in nutrient availability; (2) applicable to the analyses of a wide spectrum of nutrients (Ca, $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$, and DOC), and (3) capable of utilizing one extractant for cations and another for anions which can be successively leached through a single soil core. These criteria were established in a preliminary experiment involving various extractants and volumes. This experiment was followed by a comparison of three extraction techniques, using soil sections which were treated with heavy metals.

4.2.3.1 Materials and Methods

Soil Preparation

Soils from a mesic hardwood forest (Emory silt loam) and managed pasture (Captina silt loam) were collected from the Oak Ridge Reservation (Table 4.1). All soil cores (5 cm diam. x 5 cm depth) were excised with a steel coring device. Aboveground plant parts were removed from each grassland soil core by cutting stems at the soil surface.

Intact soil cores were constructed by encasing undisturbed cores in heat-shrinkable polyvinyl chloride plastic sheeting. A reservoir for containing 50 ml of extracting solution was made by extending the PVC sheeting 5 cm above the soil surface. Free drainage was allowed from the base of the cores.

Homogenized soil columns were prepared from oven-dried 5- x 5-cm soil cores which were pulverized and thoroughly mixed. The homogenized soil was then loosely packed into polyethylene cylinders (5 cm diam. x 10 cm depth). Each cylinder was fitted with fine-mesh nylon screening on the base to prevent particulate loss.

Intact and homogenized soil columns were placed in a Plexiglas assembly designed to hold the columns in place and to direct leachate from the columns into collection beakers. This assembly allowed convenient leaching of 20 to 30 soil columns simultaneously. After collection, all leachate samples were centrifuged at 1000 g's for 20 min prior to analysis for nutrients.

Soil slurries were prepared by the conventional method of mixing 2.5 and 5.0 g soil with 50 and 25 ml of 0.5 M NaHCO_3 and 0.5 M KCl ,

respectively (Soil Science Society of America 1967). The mixtures were agitated for 30 min to ensure thorough mixing and subsequently centrifuged to remove the extractant solution.

Chemical Analysis

Standard Autotechnicon techniques were used for analysis of $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, and $\text{PO}_4\text{-P}$. Calcium analyses were performed by flame atomic absorption. Dissolved organic carbon was determined by digestion of the leachate solutions and subsequent infrared analysis of evolved CO_2 .

Analysis of extractants. Three extractant solutions for intact cores were evaluated in terms of the operational criteria previously described. Experimental treatments were comprised of duplicate intact soil cores of Captina and Emory silt loam soil from grassland and forest ecosystems, respectively.

All intact soil cores were constructed by the previously described procedure. The cores were initially leached with 400 ml of distilled water to remove nutrients and soil colloids solubilized by the excision process. Volumes of 200 ml of extractant (0.5 M KCl , NH_4OAc , or NaHCO_3) were then leached through each core (Table 4.15). This volume was selected to approximate a soil:solution ratio of one. Soil core weights varied from 180 to 200 g.

The feasibility of successive extractions from a single soil core was evaluated using KCl and NaHCO_3 for extraction of cations and anions, respectively (Table 4.15). For this test, soil cores were successively leached with 400 ml distilled water, 200 ml of 0.5 M KCl , 200 ml distilled water, and 200 ml 0.5 M NaHCO_3 .

Table 4.15. Extraction of nutrients from intact soil cores using various extractant solutions [$\mu\text{g/g}$ ($\bar{X} \pm \text{S.E.}$)] (Jackson and Hall, submitted)

Extractant	Soil type	Ca	NH ₃ -N	NO ₃ -N	P ₀₄ -P	DOC
0.5 M <u>KCl</u>	Captina	611(98)	2.8(1.4)	0.03(0.002)	0.30(0.01)	18.1(2.1)
	Emory	707(45)	2.3(0.5)	0.04(0.002)	0.02(0.01)	14.2(1.3)
0.5 M <u>NH₄OAc</u>	Captina	445(300)		0.02(0.005)	0.08(0.05)	
	Emory	541(30)		0.02(0.0)	0.05(0.01)	
0.5 M <u>NaHCO₃</u>	Captina	16.5(1.1)	1.6(0.5)	0.09(0.01)	0.32(0.09)	230(70)
	Emory	16.6(1.8)	2.0(0.1)	0.15(0.1)	0.40(0.2)	195(36)
0.5 M <u>NaHCO₃</u> ^a	Captina	17.2(2.7)	1.4(0.01)	0.09(0.01)	0.26(0.02)	87(8)
	Emory	11.6(1.3)	3.7(0.8)	0.07(0.005)	0.18(0.04)	89(21)

^aThese soil cores were previously extracted with 0.5 M KCl.

Comparative extraction techniques. Nutrients extracted from intact and homogenized soil cores were compared to those extracted from slurried soil. Intact and homogenized cores were successively leached with 200 ml of 0.5 M KCl (for extraction of Ca and $\text{NH}_3\text{-N}$), 200 ml distilled water, and 200 ml of 0.5 M NaHCO_3 (for extraction of $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$, and DOC). Slurried soil samples were prepared by the above procedure. Soil samples extracted with KCl and NaHCO_3 were analyzed for Ca and $\text{NH}_3\text{-N}$ and for $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$, and DOC, respectively. Triplicate extractions were made of Captina and Emory silt loam soils for all three techniques.

Sensitivity analysis. Soil cores for this experiment were excised from six enclosed blocks (microcosms) of intact Emory silt loam soil. Each microcosm contained one red maple seedling (Acer rubrum), approximately 2 m high, with associated herbaceous ground cover. Three of the microcosms were treated with primary lead smelter stack emissions (equivalent to 12 mg Pb/cm^2) while three served as controls. The microcosms were maintained in a greenhouse for 20 months prior to the excision of soil cores.

Duplicate intact soil cores (5 cm diam. x 10 cm depth) were excised from each microcosm. Each core was divided at a depth of 5 cm to provide a depth-profile analysis of the soil. Cores from each microcosm were encased in shrinkable PVC for leaching with extractants. The duplicate core was dried and ground for extraction using the soil slurry technique. Extractions and nutrient analyses were done as in the previous experiment.

4.2.3.2 Results and Discussion

Analysis of Extractants

Nutrient cations (Ca and $\text{NH}_3\text{-N}$) were extracted with 0.5 M KCl and NH_4OAc (Table 4.15). The extractants were chosen on the following basis: 0.5 M KCl is commonly used for extraction of $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$, and 0.5 M NH_4OAc is commonly used for extraction of exchangeable nutrient cations. Potassium chloride was chosen as the best extractant for Ca and $\text{NH}_3\text{-N}$ because: (1) the amount of Ca extracted from the tested soils was comparable to that extracted by NH_4OAc , and (2) extractable $\text{NH}_3\text{-N}$ could not be determined using NH_4OAc as an extractant. In most cases, concentrations of nutrient anions ($\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$, and DOC) extracted with KCl and NH_4OAc were 10 fold lower than the same nutrients extracted with NaHCO_3 (Table 4.15). Thus, KCl and NH_4OAc were not used in subsequent experiments for extraction of nutrient anions.

Nutrient anions were extracted with 0.5 M NaHCO_3 (Table 4.15). This extractant removed high concentrations of $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$, and DOC (expected due to its high alkalinity, $\text{pH} = 8.3$), but was clearly ineffective in removing Ca and $\text{NH}_3\text{-N}$.

A successive leaching technique using KCl followed by NaHCO_3 was necessary to extract all nutrients from a single soil core. This criterion was based on the fact that in some experiments only one soil core is available for the extraction of all nutrients. Therefore, a procedure was adopted whereby a soil core was leached with 200 ml of KCl extractant, rinsed (to remove excess KCl) with 200 ml of distilled water, and subsequently leached with 200 ml of NaHCO_3 extractant.

Extraction of $\text{PO}_4\text{-P}$ and DOC was lowered when NaHCO_3 was leached through the cores after 0.5 M KCl and distilled water (Table 4.15). This indicates that a significant loss of these nutrients occurred from the extraction with KCl. However, the advantages offered by the successive leaching technique outweighed the loss of $\text{PO}_4\text{-P}$ and DOC. Thus, the successive leaching of soil cores with 0.5 M KCl, distilled water, and 0.5 M NaHCO_3 was adopted as the standard method for the following experiments. Extracts of KCl were analyzed for Ca and $\text{NH}_3\text{-N}$ and extracts of NaHCO_3 were analyzed for $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$, and DOC.

Comparative Extraction Techniques

All nutrients with the exception of DOC were extracted in much greater quantities using the slurry technique (Fig. 4.3). The high soil-to-solution ratio (1:5 for KCl and 1:50 for NaHCO_3) and the dispersion of soil colloids may account for these results. Values obtained by this technique are generally regarded as the total exchangeable or available amount of nutrients in the soil system (Soil Science Society of America 1967).

Lower quantities of nutrients were extracted using the leaching technique for intact and homogenized soil cores. The soil-to-solution ratio was approximately 1:1. However, the difference in soil-to-solution ratios did not account for the greater amounts of nutrients extracted by the slurry technique.

The volume of extractant solutions (KCl and NaHCO_3) was 200 ml for this technique. Further analysis showed that up to 500 ml of solution could be leached through the soil cores before nutrient concentrations were diminished in the effluent solutions. Therefore, the values

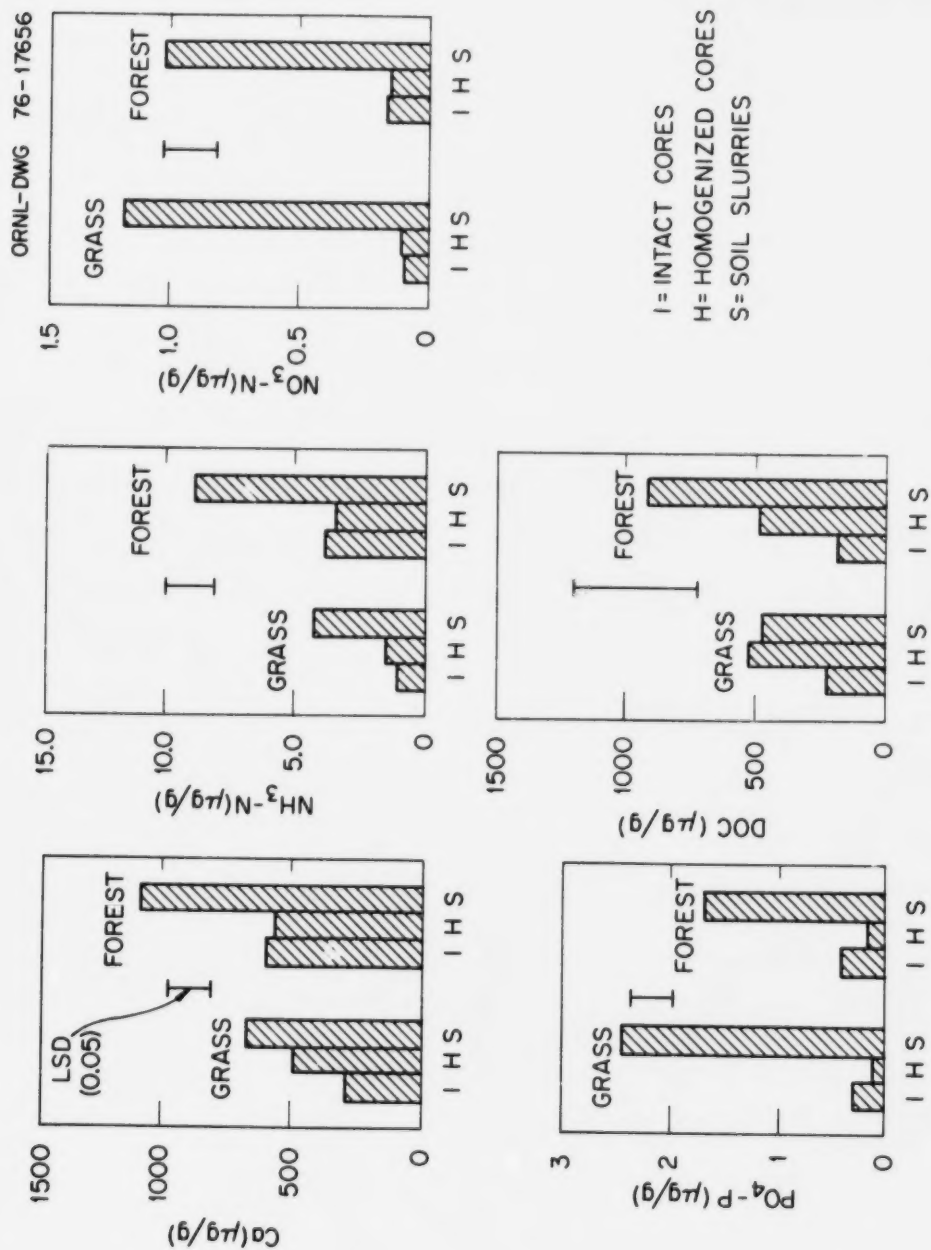


Figure 4.3. Nutrient pools extracted from Emory and Captina soils by leaching intact and homogenized soil cores, and by the slurry method (Jackson and Hall, submitted).

reported for intact and homogenized cores are interpreted as an index of available or exchangeable soil nutrients and not quantitative estimates of their gross size.

Sensitivity Analysis

The sensitivity of the intact soil core and soil slurry techniques was compared by extracting nutrients from microcosms which were treated with Pb smelter emissions (Fig. 4.1). Results clearly show that levels of Ca, $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, and DOC to a 5-cm depth extracted by the intact method were decreased from the application of smelter emissions. Identical results were obtained for $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, and DOC to a 10-cm soil depth. These data corroborate results showing increased gaseous $\text{CO}_2\text{-C}$ losses from the soil surface in addition to increased nutrient losses via soil leachate from the Pb-treated microcosms.

Analysis of slurried soil samples did not distinguish between controls and Pb-treated microcosms for all nutrients. In addition, all nutrient pools extracted by this method except for Ca and $\text{NH}_3\text{-N}$ were a factor of 10 greater than those extracted by the intact technique. These results are similar to the previous experiment (Fig. 4.1).

The significance of this experiment is that extraction of gross nutrient pools by the slurry technique was not sensitive to changes in nutrient availability resulting from application of Pb smelter emissions. However, the method of extracting intact soil cores was sensitive to this effect.

In addition to indicating nutrient losses, the intact leaching method distinguished differences between nutrient pools at 5 and 10 cm soil depth. Extraction with the intact soil core technique indicated

greater nutrient pool size at a 5-cm depth for all nutrients except $\text{PO}_4\text{-P}$ and $\text{NH}_3\text{-N}$. No differences were detected using the slurry technique.

The detection of soil nutrient loss by the intact-core leaching technique provides additional evidence that the application of heavy metals affects nutrient cycling in forested ecosystems. Remaining questions are: How much and at what rate are the soil nutrient pools depleted? Will a loss of nutrients result in decreased autotrophic and microbial growth? The answers to these questions lie in the degree of heavy metal toxicity to these organisms. If growth is inhibited by heavy metal toxicity, then the loss of nutrients will be further enhanced, resulting in gross nutrient depletion, as in the case of Crooked Creek Watershed (Jackson and Watson 1977).

Summary

A procedure was developed for extracting nutrient pools from intact soil cores. Intact soil cores were leached with solutions of 0.5 M KCl, distilled water, and 0.5 M NaHCO_3 . In this manner, analyses were completed for Ca, $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$, and DOC from a single soil core. This technique was compared to the conventional slurry extraction methods. Results indicate that a much greater quantity of nutrients were extracted by the slurry method.

Experiments in this study were conducted on silt loam soil which was easily excised intact. No significant differences were found between nutrients extracted from intact and homogenized soil. Therefore, leaching homogenized soil cores may be feasible on soils which are not easily excised from the field.

The leaching technique was found to be more effective than the slurry method in detecting nutrient losses from Pb-treated microcosms. Results from this experiment further substantiated the effects of heavy metals on nutrient cycling in forested ecosystems.

Ultimately the leaching technique may be incorporated into a protocol for assessing the potential toxicity of new chemicals to terrestrial ecosystems.

4.2.4 Hollow Fiber Membranes for Detecting Microbial and Nutrient Cycling Effects of Contaminants

This experiment tested the applicability of polycarbonate hollow fibers within microcosms for collection of soil solution at various depths to monitor contaminant and essential element concentrations in solution. We also examined the feasibility of glass manifolds (fitted with fibers for collection of litter and soil water in field plots) to validate microcosm transport data.

The specific objectives of this study were to: (1) compare the nutrient composition of soil water extracted from intact soil cores by Diaflo^(R) hollow fibers to that collected by porous ceramic cup lysimeter and soil leachate; and (2) evaluate the sensitivity of hollow fiber and lysimeter extraction techniques to discriminate soil depth in terms of dissolved nutrients in soil water from intact grassland soil cores.

4.2.4.1 Materials and Methods

Three intact soil cores (30 cm diam. x 25 cm depth) were excised from a managed pasture on the Oak Ridge Reservation. All intact soil cores were fitted with a lysimeter and a bundle of hollow fibers

mounted horizontally at 10- and 20-cm depths (Fig. 4.4). Each bundle of four hollow fibers was joined at one end with glass tubing (9.3 cm diam., 2 cm length) and plugged with RTV 3140 clear silicone rubber adhesive. The opposite end was secured in a similar fashion, but was left open, allowing a tube connection to pull suction from a vacuum pump. The fiber bundles were installed by tunneling a passage through the side of the core with a metal tube (0.3 cm diam., 40 cm length) and threading the fibers through. The metal tube was then removed. The entire core was wrapped in polyethylene sheeting and encased inside a plastic tub with concrete for support and insulation. A leachate port was constructed at the bottom of each soil core. Polyethylene tubing (0.63 cm diam.) was imbedded in the concrete at the 10-, 20-, and 25-cm depths for separate connections from the fiber bundles and the leachate port to the outside of the tub. The soil cores were also fitted with Irrometers^(R) at 10- and 20-cm depths (Fig. 4.4). The Irrometers^(R) were previously calibrated to indicate percent soil moisture.

Using estimates of soil core weight and measurements from the installed Irrometers^(R), the soil cores were watered to 130% field capacity (F.C. = 25% soil moisture) once per week. The soil core was allowed to equilibrate to field capacity. Water volumes greater than field capacity (estimated to be 795 ml H₂O) drained through the leachate port and were collected for chemical analysis. Subsequently, a small vacuum pump was attached with a sample collection vial to the fiber bundle connections outside the tub (Fig. 4.4). A vacuum of 25 cm Hg was applied for 5 to 6 hr to obtain 15 to 30 ml of solution from soil surrounding each fiber bundle. Concurrently, porous cup lysimeters

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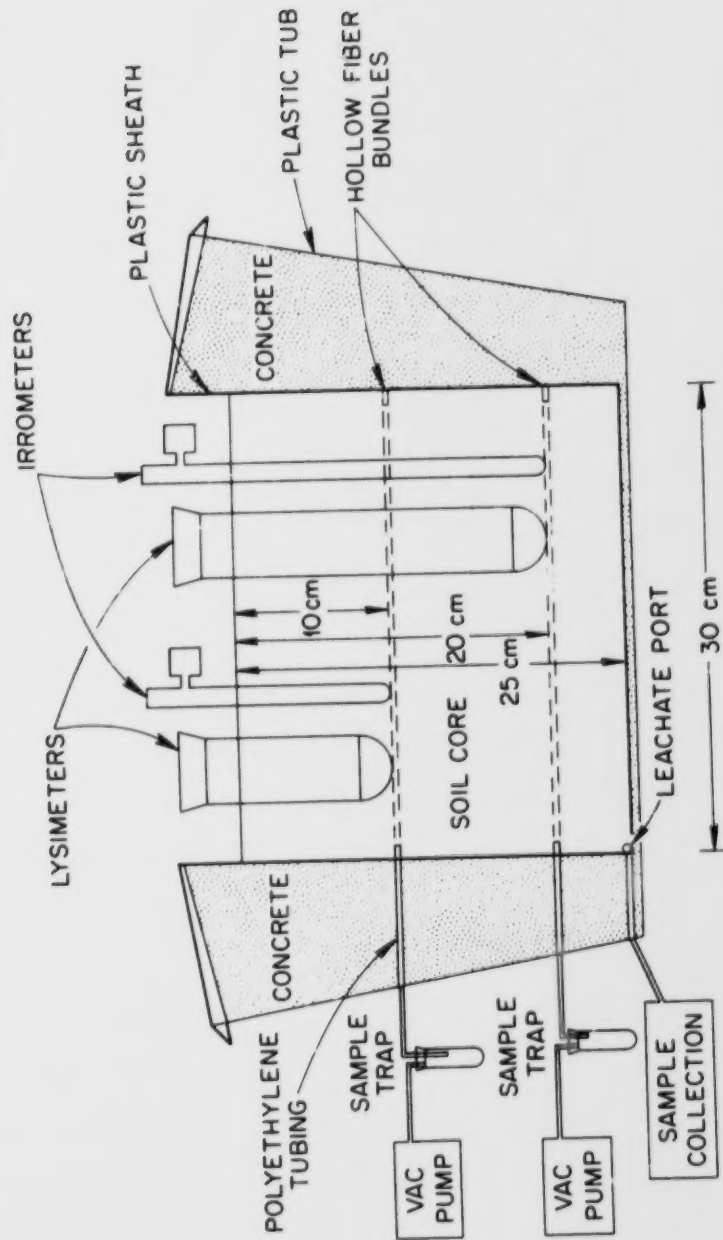


Figure 4.4. Schematic diagram of an intact soil core containing devices for extracting in-situ soil solution.

were evacuated to a tension of 0.8 atm. Approximately 10 to 30 ml water were pulled into the lysimeters through the porous cups after 5 to 6 hr of extraction. Solutions were pumped from the lysimeters into sample containers at the end of the collection period. Five successive weekly collections of soil water were made in this manner. All solutions were subsequently passed through 0.4- μ Nuclepore membrane filters. Concentrations of Ca and Mg were determined by flame atomic absorption. Automated Technicon^(R) procedures were used for $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ determinations. Electrical conductivity (EC) measurements were made on all samples.

4.2.4.2 Results and Discussion

Concentrations of Ca and Mg and mmhos/cm EC in soil solutions extracted by lysimeter and hollow fiber techniques were significantly greater ($P \leq 0.05$) at 10-cm than at 20-cm soil depths (Table 4.16). However, concentrations of $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ were the same between these depths. Close agreement was found in EC and all nutrient concentrations between solutions extracted with lysimeters and hollow fibers. In addition, no significant difference of nutrient concentrations except $\text{NO}_3\text{-N}$ was noted between soil solutions extracted at 20 cm by lysimeters, hollow fiber techniques, and soil leachate collected at 25 cm (Table 4.16). Screening of $\text{NO}_3\text{-N}$ by lysimeters and hollow fibers was indicated from these results (Table 4.16). Closer scrutiny is necessary to determine if this effect is real or a unique characteristic of this experiment.

Samples of sufficient quantity for chemical analyses were extracted by hollow fiber and lysimeter techniques in reasonably short periods of

Table 4.16. Mean nutrient concentrations ($\mu\text{g/ml}$) of soil solutions extracted from 10- and 20-cm depths with hollow fibers and lysimeters ($\pm 1 \text{ S.E.}$, $n=10$)

Depth (cm)	Mode of extraction	$\text{NO}_3\text{-N}$	Ca	Mg	$\text{PO}_4\text{-P}$	Electrical conductivity (mhos/cm)
10	Lysimeter	0.11(0.002)	7.89(1.04)	1.58(0.20)	0.005(0.002)	0.072(0.007)
10	Fiber bundle	0.017(0.004)	9.20(1.09)	1.45(0.22)	0.013(0.008)	0.073(0.006)
20	Lysimeter	0.008(0.001)	5.24(0.39)	1.09(0.07)	0.005(0.002)	0.052(0.003)
20	Fiber bundle	0.013(0.003)	5.23(0.57)	1.06(0.10)	0.005(0.004)	0.054(0.005)
25	Leachate port	0.024(0.004)	4.66(0.65)	1.11(0.14)	0.018(0.006)	0.049(0.004)
LSD Values ($P \leq 0.05$)						
Comparison		$\text{NO}_3\text{-N}$	Ca	Mg	$\text{PO}_4\text{-P}$	Electrical conductivity
10 cm vs 20 cm (fiber, lysimeter)		0.005	1.53	0.270	0.008	0.010
20 cm vs 25 cm (fiber, lysimeter, port)		0.008	1.079	0.194	0.012	0.006

time. The solutions were clear and no visible residues were left on the Nuclepore membrane filters after solutions were passed through. Hollow fibers were flexible, easy to manipulate, and resistant to breakage. The fiber bundles continued to extract water at comparable rates throughout the experiment despite cyclic wetting and drying conditions. Clogging was not a problem with either hollow fibers or porous ceramic-cup lysimeters.

4.2.4.3 Summary

This experiment demonstrates that porous ceramic-cup lysimeters and hollow fibers are viable techniques for in situ extraction of soil solution. Hollow fiber and porous ceramic-cup lysimeter techniques did not affect EC and concentrations of Ca, Mg, and $\text{PO}_4\text{-P}$, and were sensitive to differences in dissolved nutrients with respect to soil depth. However, apparent screening of $\text{NO}_3\text{-N}$ was found to occur with both techniques.

Hollow fibers are considered more useful for extraction of water from greenhouse pots and intact soil cores because this technique displaces a smaller volume of soil compared to the cup lysimeter. Cup lysimeters are more useful for field sampling.

Laboratory Soil Water Extraction

Amicon^(R) hollow fibers can be used (in pairs) in intact soil microcosms. They can be inserted as described above and monitored on a regular schedule. Preliminary studies have shown that fibers can be reused after long intervals of inactivity and can be successfully transferred to new experiments.

Field Use of Fibers

Manifolds of glass might be useful in adapting Amicon^(R) fibers to field use, especially to extract litter solution entering soil (Fig. 4.5). The manifolds were used by placing one unit at the interface of O1 and O2 litter and a second at the litter-soil interface. A moisture-activated switch engages a vacuum pump which applies tension to fibers. Water extracted from litter percolate is collected in a bottle attached to the vacuum pump.

4.2.5 The Effect of Substrate on Elemental Transport Through Terrestrial Microcosms

The objective of this experiment was to determine the effect of substrate on transport of essential elements through terrestrial microcosms. Substrates used for microcosms are not now standardized. Microcosm results have been reported using soil, sand, synthetic, and mixed substrates. Substrate may significantly influence parameters estimated by microcosm screening techniques. Essential element transport through substrates used in microcosm units should furnish a good test of substrate effect because (1) essential element transport is well documented in terrestrial ecosystems, and (2) rates of essential element transport have been shown to increase under perturbation.

The experimental design was randomized complete block design with three treatments (intact soil, homogenized soil, and sand/soil substrates), with two blocks with three replicates each. After encasement, microcosms were equilibrated for three weeks and then monitored for 118 days without treatment. For 18 successive weeks leachate was collected, following rainfall additions in excess of soil water-holding capacity.

ORNL-DWG 77-3837

221

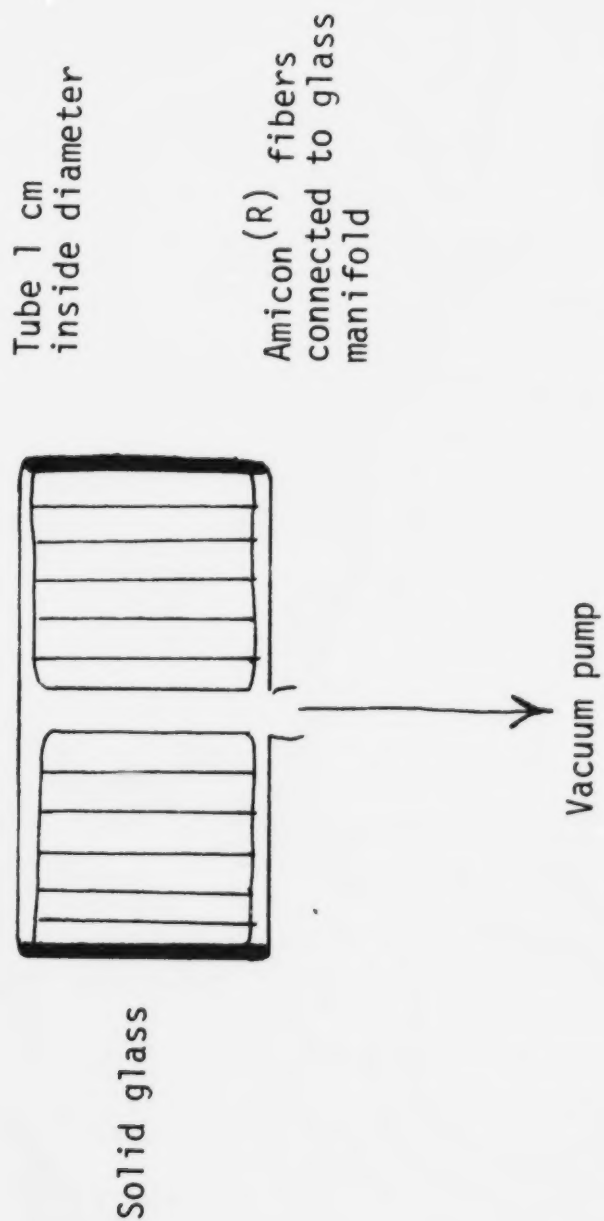


Figure 4.5. Schematic of glass manifolds for field use of polycarbonate hollow fibers.

Concentrations of essential elements in rainfall and leachate were then determined. Elemental transport was calculated from concentration data multiplied by the leachate volume from each microcosm, respectively.

4.2.5.1 Results and Discussion

Substrate had a significant effect on the amounts, rates, and patterns of essential elemental transport through terrestrial microcosms.

Cumulative Transport

Substrate significantly affected total transport of essential elements from microcosms over the 118-day experiment. Table 4.17 summarizes the total loss from each substrate of Ca, K, DOC, $\text{PO}_4\text{-P}$, $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, Na, and Mg. For each element examined there was a significant difference in transport between intact soil and either or both of the other substrates. There was a significant block effect within homogenized soil microcosms for all elements measured. Transport of Ca, Mg, Na, and DOC was greater in one of two blocks of homogenized substrate and the sand/soil mixture. Potassium transport was greatest from sand/soil, least from homogenized soil. Ammonia-N transport was greater from intact soil than that from other substrates. Ammonia-N loss averaged 59% nitrate-N loss weekly from intact soil microcosms over the interval. Phosphate-P and $\text{NO}_3\text{-N}$ transport were most variable of any element examined. Block A homogenized soil microcosms transported more $\text{PO}_4\text{-P}$ but less $\text{NH}_3\text{-N}$ than intact soil. Maximum $\text{NO}_3\text{-N}$ transport was observed in homogenized soil, Block B. Sand/soil microcosms transported significantly less $\text{PO}_4\text{-P}$ and $\text{NO}_3\text{-N}$ than intact soil.

Table 4.17. Cumulative elemental transport (mg) through terrestrial microcosms of three substrates over a 118-day experiment

Substrate (replicates, dates)	Elemental transport (Total, 118 days)						
	Ca	K	Mg	Na	NH ₃ -N	NO ₃ -N	P ₀₄ -P
Intact (6, 18)	3.68	3.42	0.57	0.40	0.420	0.868	0.015
Homogenized Block A (3, 18)	6.13 ^a	2.66 ^a	0.74 ^a	0.51	0.194 ^b	0.242 ^b	0.058 ^b
(3, 18) Block B	3.96	2.52 ^a	0.57	1.80 ^a	0.139 ^b	1.505 ^{b*}	0.011
Sand-soil (6, 18)	4.79 ^a	6.99	0.79 ^a	0.80 ^a	0.145 ^b	0.142 ^b	0.003 ^a
							10.59 ^a

^aSignificantly different from intact $P \leq 0.05$.

^bSignificantly different from intact $P \leq 0.01$.

Transport Rates

Substrate significantly affected rates of elemental transport through terrestrial microcosms (Table 4.18). For example, rates of DOC, Ca, Mg, and Na were significantly less from intact soil than those from other substrates.

Rates of elemental transport were quite constant in intact soil microcosms through the 18-week experiment. However, rates of elemental transport were erratic from homogenized soil and sand/soil substrates through time. Too, variation in transport rates among replicates within substrates was often 2 to 5 fold. For example, $\text{NO}_3\text{-N}$ and $\text{NH}_3\text{-N}$ loss rates were radically different between blocks of homogenized soil microcosms. Over the 18-week experiment, rates of $\text{NH}_3\text{-N}$ loss decreased within one block but increased within the other. The converse was observed in $\text{NO}_3\text{-N}$ transport. Rates of DOC and Ca transport increased with time in four of six sand/soil microcosms but remained relatively constant from the remaining two replications. At the end of the experiment, cumulative DOC and Ca transport was twice as great from this group of four replicates of sand/soil microcosms as that from the others. There was no apparent cause for these differences among replicates within substrate groups. These differences were not detected in CO_2 efflux measured concomitantly and were minimal for transport of some elements such as K and Mg.

Replicability

The replicability of observations made over the 18 weeks on elemental transport through intact soil microcosms was equal to and/or greater than that of other substrates. Mean coefficients of variation

Table 4.18. Rates of essential element transport (mg/microcosm/week) through terrestrial microcosms constructed of three substrates (N = 18 weekly observations)

Substrate	Elemental transport $\bar{x}$ (S.E.)							
	Ca	K	Mg	Na	NH ₃ -N	NO ₃ -N	PO ₄ -P	DOC
Intact	204.5 (18.6)	190.1 (9.3)	31.5 (2.9)	22.5 (1.3)	27.6 (3.4)	47.7 (4.6)	0.84 (0.17)	219.4 (16.9)
Homogenized	284.3 (13.8)	134.6 ^a (4.8)	36.4 (1.8)	63.4 ^a (6.5)	58.2 (6.8)	48.5 (6.4)	2.03 ^a (0.31)	496.8 ^a (45.5)
Sand-soil	298.9 (27.2)	286.2 ^a (45.2)	39.8 (3.5)	44.3 ^a (2.6)	7.9 ^b (1.1)	7.7 ^b (1.7)	0.48 ^a (0.08)	945.1 ^b (135.6)

^aSignificantly different from intact $P \leq 0.05$.

^bSignificantly different from intact $P \leq 0.01$.

(C.V.) over the 18-week experiment were calculated for essential elements studied. Mean C.V. was not significantly different among substrates for Ca, Mg, $\text{PO}_4\text{-P}$, and total P. Mean C.V. was significantly less in intact soil than that in homogenized soil for DOC and Na but was significantly greater for K. Mean C.V. of sand/soil microcosms was significantly less than that of intact soil for $\text{NO}_3\text{-N}$ and $\text{NH}_3\text{-N}$ transport but was significantly greater than that for transport of K and $\text{SO}_4\text{-S}$.

Greater insight into the replicability of substrate elemental transport was provided by calculating the probability (Z) that successive measurements of transport of any element from an individual microcosm unit differ by greater than 100% (Table 4.19). In no case was the probability greater for intact soil microcosms than that for other substrates ($P \leq 0.05$). Three elements showed a significant increase in Z in homogenized soil microcosms: $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, and Mg. Sand/soil microcosms showed the greatest probability of change ($> 100\%$) between successive observations. The Z values were significantly greater in sand/soil microcosms than those in intact soil for six of the eight elements measured: DOC, $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, Mg, K, and Na. Neither Ca nor $\text{PO}_4\text{-P}$ showed significant differences among substrates. We, therefore, conclude that replicability was greatest in microcosms constructed of intact soil.

4.2.6 Functional Complexity and Microcosm Stability

To rely on microcosms as useful screening tools for environmental impact assessment, it is necessary to understand their long-term response to extraction and maintenance under controlled environmental

Table 4.19. Probability (Z) that successive observations of elemental transport through any microcosm unit differ by > 100% (N = 17/replicates)

Substrate	Z or probability (S.E.)						
	DOC	Ca	NH ₃	NO ₃	Mg	PO ₄	Na
Intact	0.24 (0.06)	0.24 (0.06)	0.29 (0.06)	0.35 (0.03)	0.12 (0.06)	0.65 (0.06)	0.12 (0.06)
Homogenized	0.35 (0.12)	0.18 (0.06)	0.47 ^a (0.06)	0.53 ^a (0.06)	0.24 ^a (0.06)	0.71 (0.06)	0.18 (0.06)
Sand-soil	0.47 ^a (0.11)	0.41 (0.11)	0.56 ^a (0.11)	0.65 ^a (0.06)	0.41 ^a (0.06)	0.65 (0.06)	0.24 ^a (0.06)

^aSignificantly greater than intact substrate at $P < 0.05$.

conditions. It is also important to ascertain the stability of each microcosm and to determine if differences in stability are related to the functional complexity that is intrinsic to each microcosm system.

The objectives of this experiment were: (1) to develop valid procedures for encasing terrestrial microcosms and to determine if materials used have any effect on parameters being measured, (2) to develop techniques for measuring total terrestrial microcosm metabolism, and (3) to develop a method of determining the relative functional complexity of each microcosm and to investigate its relationship to stability.

A central hypothesis in ecology is that system stability is in some way associated with complexity (e.g., MacArthur 1955, May 1973). The issue has been examined in some detail through mathematical models. In addition, limited data on interacting populations have been collected which in some cases support a stability-complexity relationship. In an effort to extend the experimental evidence on this theme to the ecosystem level, a stability experiment using old field microcosms was performed.

Correlated questions also were investigated: (1) how do terrestrial microcosms behave under regulated environmental conditions over long periods of time without being perturbed; (2) what is a reasonable equilibrium time for microcosms of this type; and (3) how will seasonality affect microcosm results. These objectives and correlated questions relate directly to project goals of determining the reliability and utility of microcosms as reasonable screening tools for transport, fate, and effects of toxic substances.

4.2.6.1 Materials and Methods

Thirteen intact cores were extracted from the approximate center of an old field located on the U. S. Department of Energy reservation at Oak Ridge, Tennessee.

Each microcosm, with litter and autotrophs intact, was trimmed to predetermined size (15 x 10 cm) and sleeved with 0.10-cm thick polyvinyl chloride (PVC). A Plexiglas disk, with leachate port attached, was pressed to the bottom of the soil profile, and the PVC heat shrunk until taut. The encased microcosm was placed in a small polyethylene pot, medium grain quartz sand packed between the PVC and pot, and the microcosm placed in the environmental chamber (Fig. 4.6).

Complexity of each microcosm was characterized by analyzing system dynamics evident in CO_2 efflux measurements. Carbon dioxide efflux is an ecosystem-level variable which to some degree integrates the dynamics of all system components. Hourly measurements of CO_2 efflux were obtained for 2050 hr under controlled environmental conditions using infrared gas analysis techniques. Sample data are displayed in Fig. 4.7. The CO_2 time series is dominated by cyclic dynamics. The power spectral density was calculated for the CO_2 time series for each microcosm using standard methods (Blackman and Tukey 1958). This spectral analysis elucidates the relative importance of cyclic components of different periods required to decompose the time series into sinusoids. It is assumed that each significant cyclic component (i.e., peaks in the power spectral density) was the result of functional interactions (e.g., predator-prey interaction) in the microcosm, so that the number of peaks in the power spectral density is hypothesized to be a measure of the dynamic or functional complexity of the system.

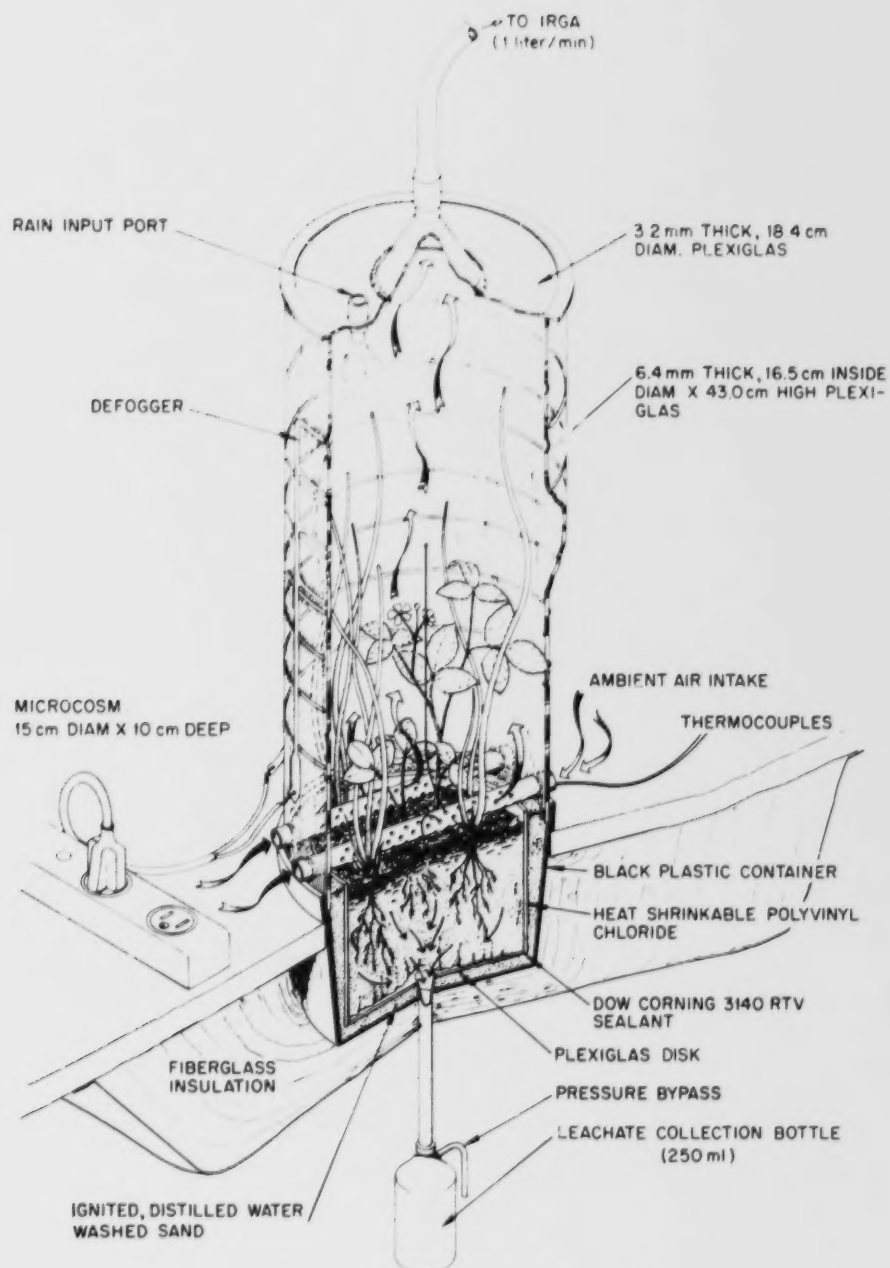


Figure 4.6. Cross-section of old-field microcosm showing two system-level monitoring points of CO₂ efflux and nutrient leachate.

ORNL-DWG 77-13383

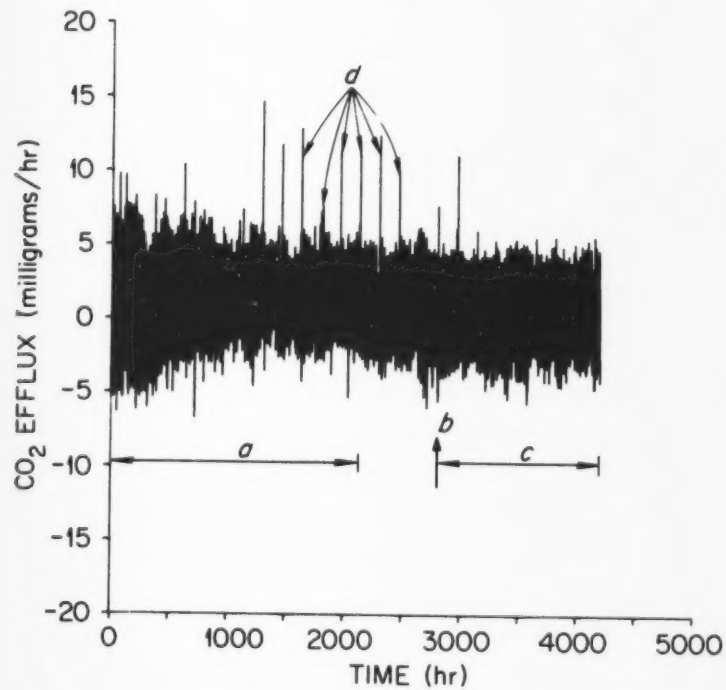
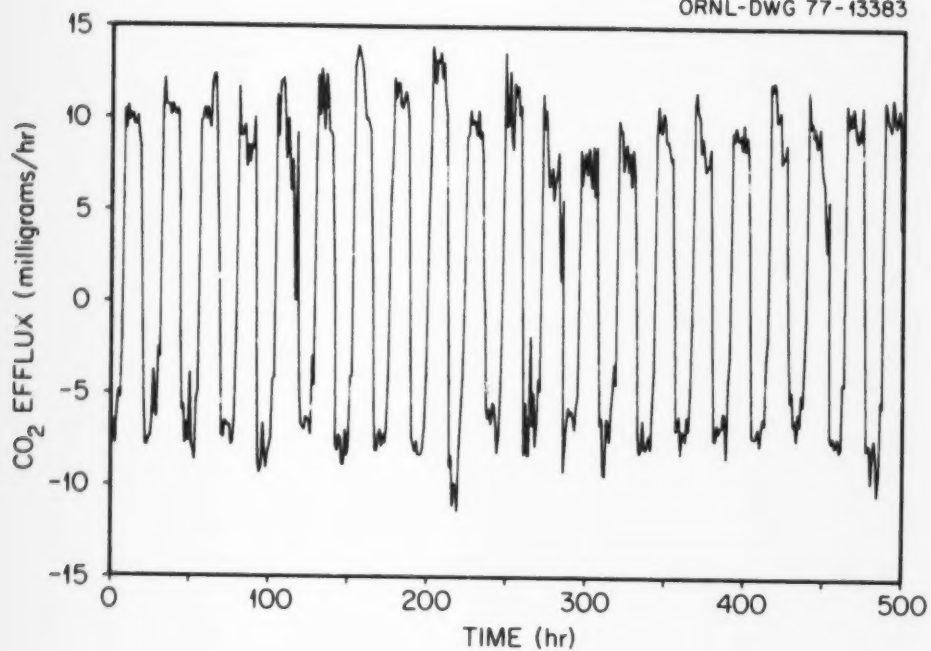


Figure 4.7. Example of time series of CO₂ efflux from pasture microcosms.

The stability of each microcosm was established by performing a perturbation experiment using cadmium in toxic concentrations. A dose per unit area of 0.4 mg/cm^2 was chosen. The response of the microcosms to this perturbation was monitored by weekly measurement of the export of Ca in leachate. Typical response of this variable to the perturbation is illustrated in Fig. 4.8. Stability, in the sense of resistance and ability to recover from perturbation, was summarized by calculating the area under the Ca response curve. Stability ranking by this measure and complexity are summarized for each microcosm in Table 4.20.

Encasement Procedures

Encasement of microcosms in epoxy proved to be an unreliable method due to both the chemical nature and the lack of predictability of hardening of the epoxy. The epoxy was found to infiltrate the entire soil column and harden incompletely. In addition, tests of leachability of epoxy were made. These tests indicated that the epoxy leached dissolved organic carbon over a long period of time. The amount of leaching was directly related to the relative hardness of epoxy. Thus, a new encasement technique was developed and tested for this type of intact microcosm.

The new encasement method follows the procedure described below:

- (1) Place a 15.5-cm diam. sleeve of polyvinyl chloride (PVC) around the 15-cm diam. microcosm.
- (2) Place a 0.64-cm thick x 15 cm-diam. Plexiglas disk with a 1.23-cm diam. hole in the center, inside the PVC sleeve.

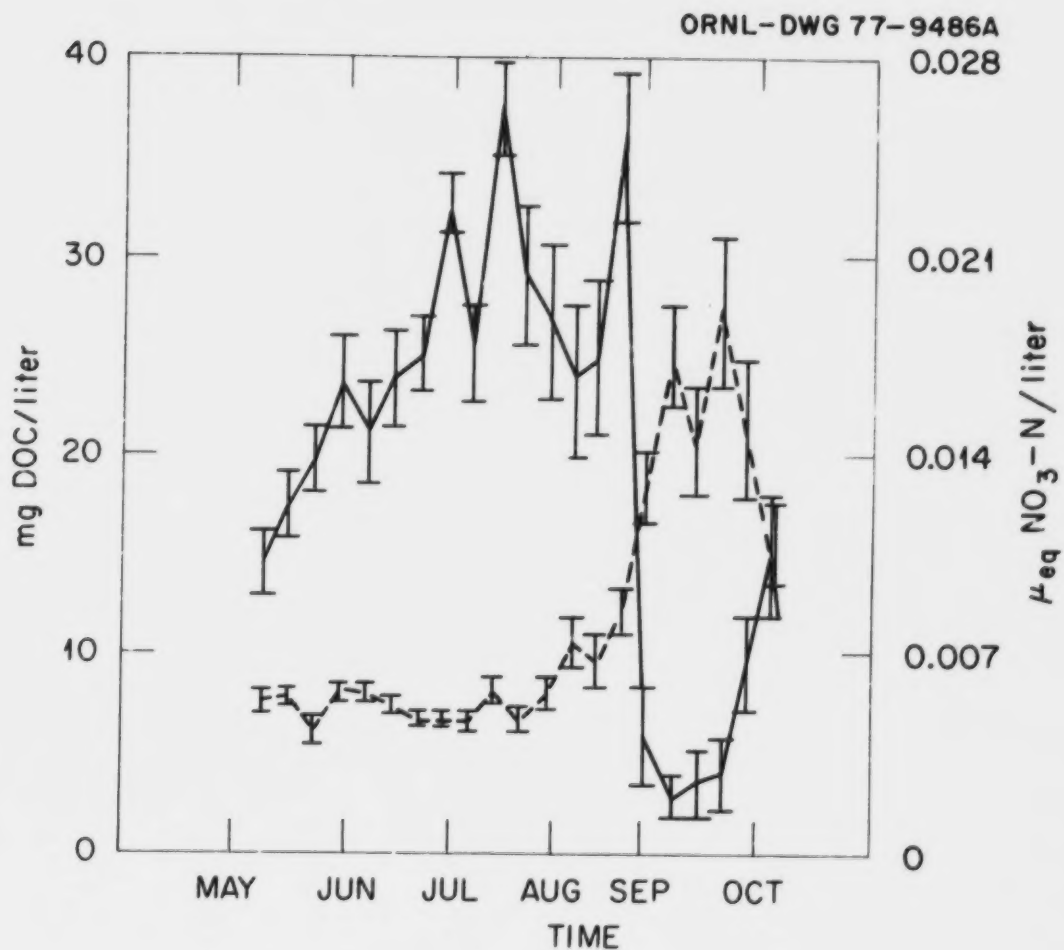


Figure 4.8. Mean DOC (-----) and $\text{NO}_3\text{-N}$ (—) concentration in leachate (± 1 S.E., $N = 13$).

Table 4.20. Rankings of functional complexity and ecosystem stability based on monitoring of CO₂ and total Ca export in soil leachate

Microcosm number	Functional complexity		Ecosystem stability	
	Peaks	Rank	Ca loss ^a	Rank
1	10	8.5	289.5	3
2	12	5	203.6	2
3	13	3.5	315.5	5
4	11	6.5	379.3	7
5	16	1.5	133.3	1
6	16	1.5	314.0	4
7	9	10	434.4	9
8	10	8.5	543.5	10
9	13	3.5	353.0	6
10	11	6.5	420.6	8

^aCa loss expressed as total μeq .

- (3) Place a small bead of Dow Corning RTV sealant along the edge of the Plexiglas disk.
- (4) Heat-shrink the PVC until skin-tight around the microcosm and the edge of the Plexiglas dish.
- (5) Remove excess PVC from both top and bottom of the microcosm leaving approximately a 2.5-cm lip on top.
- (6) Tighten a hose clamp around the outside edge of the Plexiglas disk which has been covered by the PVC. This ensures bonding between the Plexiglas and PVC. Additional RTV may be applied around the edges from the bottom as needed.
- (7) Allow 24 hr for the RTV to harden, insert plastic port into hole in Plexiglas disk, and place encased microcosm into a (18-cm diam. x 14-cm high) plastic bucket painted black to reduce light transmission.
- (8) Back fill between the PVC-wrapped microcosm and container with pre-ignited quartz sand that has been washed with acid and distilled water.

Figure 4.6 shows a cross-sectional view of an encased microcosm and the cuvette designed for CO₂ monitoring.

Materials Tests

Due to the problems of leaching encountered with epoxy, all materials used for encasement have been tested for their potential effect on nutrient concentrations in the leachate. An experiment was designed to test each of the materials that potentially could come in contact with the leachate under similar pH conditions (rainwater

pH = 6.40) and constant contact over a reasonable length of time. The materials tested include vinyl tubing, leachate collection bottles, leachate ports, sand, PVC, RTV sealant, nuclepore filters, Plexiglas, and teflon. All materials were placed in 125-ml flasks (N=3) with 100 ml of distilled water (pH 6.4) and shaken at 250. Aliquots of the 100-ml solution were removed from each sample after 2, 4, and 8 days. After each sampling period, the distilled water was changed. Each sample at each time period was subsequently analyzed for Ca, DOC, and $\text{NO}_3\text{-N}$.

Analytical results, after adjusting element concentrations of each sample at each time period for distilled water concentrations as background, showed that the only encasement material likely to leach any of the nutrients analyzed is RTV coating. It was found that the RTV coating, under these rigorous test conditions, might have a small effect on the DOC concentration. However, this possible effect diminished with time. Due to the limited contact that might occur between the leachate and the RTV, this effect is judged to be insignificant. All other materials (vinyl tubing, leachate collection bottles, leachate ports, sand, PVC, nuclepore filters, Plexiglas, and teflon) did not significantly affect Ca, DOC, or $\text{NO}_3\text{-N}$ concentrations during any sampling period.

4.2.6.2 Results

Nutrient Losses

Nutrient leachate data from 13 intact old-field microcosms (15 cm diam. x 10 cm deep) containing naturally occurring autotrophs have been

compiled during an eight-month equilibration period. During this period, weekly amendments of collected rainwater (of a known volume and nutrient concentration) were added to each microcosm. Leachate volumes for each microcosm were recorded, an aliquot was filtered through a 0.45- μm Nuclepore filter, and analytical determinations for Ca, DOC, $\text{NO}_3\text{-N}$, and $\text{NH}_3\text{-N}$ were made.

Results show that there was a significant increase in the DOC concentration in early September and a corresponding decrease in $\text{NO}_3\text{-N}$ at the same time (Fig. 4.8). These results were compiled during unperturbed conditions and are postulated to be associated with the natural senescent behavior of the autotrophic component and microbial immobilization of essential nutrients during simulations of fall conditions of light and temperature. This demonstrates that seasonality, if not accounted for, might obscure results of contaminant effect on nutrient dynamics.

Microcosm Metabolism

The objective of monitoring CO_2 was to test whether or not individual temperate grassland microcosms, with the autotrophic component intact, exhibit significant differences in their functional complexity. This was ascertained through the use of power spectral density analysis of CO_2 efflux measurements. The Fast Fourier Transform algorithm was used to compute the power spectral density of the equally spaced time series of CO_2 only after the data have been detrended and smoothed. Once this was accomplished, the next step was to determine if there is a correlation between these dynamic patterns and the relative stability of each microcosm. In this case, stability

was measured as the percent deflection of nutrient losses from normal operational patterns and the length of time necessary to return to some stable state after perturbation.

Due to the complexity of the question, it was necessary to design and fabricate an automatic sampling system which would serially sample an air stream for CO_2 concentration being drawn from the enclosure of each microcosm (Fig. 4.6). The sampling system is designed to draw air from each microcosm at the rate of 1.0 liter/min continuously, with the air stream from each microcosm being sampled hourly for CO_2 concentration using a Beckman 215A Infrared Gas Analyzer (IRGA). This allows for collection of an uninterrupted, equally spaced, time series of mg CO_2/hr for all eleven microcosms. To date, the system has been operational for 130 days with > 1% downtime.

A representative example of the CO_2 time series is shown in Fig. 4.7. These were examined using power spectral density analysis for each microcosm. There was little or no difference in rhythmic behavior among microcosms as ascertained through power spectral density analysis. This interpretation was highly influenced by the magnitude of the peak associated with the diel rhythm. After fitting and removing from the data a sinusoid of this dominant frequency, we were able to detect high period patterns which occurred at low frequency. Thus, the CO_2 data indicate that all microcosms seem to be operating with the same general trends and oscillatory patterns; however, there are some subtle differences in rhythmic patterns in the low frequency regions. We conclude that power spectral density analysis is a useful analytical tool to reveal differences in functional complexity and subsequently show its relationship to the relative stability of each microcosm.

4.2.6.3 Discussion

The microcosms were rank-ordered, based on stability and complexity measures as described above. Spearman's rank correlation test was used to test the hypothesis that stability does not increase as complexity increases. The hypothesis was rejected at the 95% confidence level.

Further details of this experiment to study the relationship between complexity and stability in laboratory ecosystems may be found in Van Voris et al. (1978). The experimental verification of an increasing stability with increasing complexity hypothesis described here is of course limited. Rather than providing results which conclusively establish the relationship between complexity and stability, this experiment demonstrates clearly that the manner in which stability and complexity are characterized and the level at which experiments are performed are critical to verifying theoretical conjectures associated with stability and complexity.

4.3 SYNTHESIS OF TERRESTRIAL MICROCOSM RESULTS (Monte Ross-Todd, E. G. O'Neill, and R. V. O'Neill)

4.3.1 Introduction

The microcosm project involved more than a dozen individual experiments designed to examine the scientific basis of microcosms as screening tools. The individual experiments focused on specific problems of size, substrate, parameters to be monitored, etc. These experimental results form the main output from the project and have been documented in a series of open literature papers.

While the papers focus on results of single experiments, the number of experiments performed over the course of the project requires that

some effort be expended in looking across the suite of experiments to identify patterns that might not be identified in individual papers. The objective of the synthesis project was to compare the results of all terrestrial experiments with a view toward answering the following questions: (1) What is the minimum set of nutrient parameters which should be measured in a large-scale monitoring system?; (2) Is there a significant effect of size of the microcosms on their ability to detect ecological changes resulting from toxic substances?; (3) Is there a significant effect of soil type or soil preparation (i.e., intact versus homogenized core) on ability to detect change? These three questions provided the focus for this project and will be considered in individual subsections of this report.

Table 4.21 outlines the characteristics of the eight terrestrial experiments which formed the material for this synthesis effort. Each experiment has been designated by a code name given in the first column of the table. These names will be used for convenience throughout this section. It is clear from the table that a great variety of experimental designs were utilized and this seriously constrains the types of cross-comparisons that are possible. Thus, it was difficult to find a single factor which differed between two experiments while all other factors remained constant. In spite of the difficulties inherent in the effort, a number of interesting results were discovered.

4.3.2 Minimal Monitoring Parameters

The suite of seven chemical species which were measured through most of the terrestrial microcosm experiments (see Table 4.22) is too large a number to be practicable in a routine screening program. The

Table 4.21. Summary of microcosm experiments and corresponding parameters

	Substrate(s)	Size(s) (cm)	Location(s)	Nutrients measured	Vegetation
Grassland I	Grassland	10x10 30x25	Greenhouse and field	Ca, Mg, DOC, NO ₃ , P, K	Yes
Grassland II	Grassland- 1. Homog. 2. Intact	15x25 30x25	Slathouse and field	Ca, Mg, DOC NO ₃ , P, K	Yes
Van Voris	Grassland	15x10	Environ. chambers	Ca, Mg, K P, DOC, NO ₃ , NH ₃	Yes
Treecosm I	Forest	63x63x45	Greenhouse	Ca, Mg, K Cd, Cu, Pb, Zn	Yes
Treecosm II	Forest	63x63x45	Slathouse and field	Ca, P, NO ₃ NH ₃ , DOC, K	Yes
Washburn	Grassland and forest	5x5	Environ. chambers	Ca, Mg, K, P P, NO ₃ , NH ₃	No
O'Neill	Forest- 1. Intact 2. Homog. 3. Sand-soil	5x5	Environ. chambers	Ca, K, DOC P, NO ₃ , NH ₃	No
Cd dosage	Forest Pine stand	5x5	Environ. chambers	Ca, DOC, NO ₃	No

Table 4.22. Comparison of variability among elements leached from terrestrial microcosms. Values are coefficients of variation (S.D. x 100/mean) averaged across all treatments in the experiment. "R" indicates variability among replicates (C.V. calculated for each leaching date and then averaged). "T" indicates variability through time (C.V. calculated for each core and then averaged).^a

		Mg	Ca	DOC	K	NO ₃	P	NH ₄
Grassland I	R	57	63	65	84	126*	116*	
	T	<u>50</u>	<u>69</u>	78	<u>68</u>	144*	147*	
Grassland II	R	57	73	62	88	122*	116*	
	T	<u>46</u>	<u>60</u>	<u>62</u>	80	117*	147*	
Van Voris	R	23	27	45	52	72	113*	78*
	T	<u>61</u>	<u>51</u>	79	<u>55</u>	85	183*	96*
Treecosc I	R	36	41		50	174*	90*	
	T	<u>50</u>	<u>59</u>		161*	67*	115*	
Treecosc II	R		51	72	70	136*	103*	93
	T		<u>44</u>	<u>76</u>	<u>76</u>	131	149*	203*
Washburn	R	30	34	33	53*	52	72*	
	T	<u>54</u>	<u>53</u>	<u>92</u>	71	100*	116*	
O'Neill	R	32	35	52	42	69	81*	89*
	T	<u>55</u>	<u>59</u>	84	48	163*	169*	156
Dosage Cd	R		49	41		68*		
	T		<u>55</u>	<u>64</u>		66*		

^aThe two lowest coefficients are underlined in each comparison and the two largest are indicated by an asterisk.

first objective of the synthesis subproject was to examine all the experimental data and determine objective, analytical approaches to reducing the number of species to be considered in later applications of the soil core microcosms.

The first step was to analyze variability in the leachate data in an effort to locate chemical species which showed little variability during the experiments and would, therefore, be likely candidates for reliable, consistent indicators. Chemical species which showed themselves to be highly variable either between replicates (R in Tables 4.22 and 4.23) or through time (T in Tables 4.22 and 4.23) would be expected to be unreliable indicators of disturbance to the system. They would be unreliable because it would be difficult to differentiate random fluctuations from treatment effects. All other factors being equal, it would require fewer replicates to detect a disturbance in a chemical species with low variability between replicates.

We have calculated coefficients of variation for each of the experiments, either lumping all treatments (Table 4.22) or considering only controls (Table 4.23). Overall examination of the tables reveals a definite pattern. Calcium, magnesium, and DOC consistently showed lower variability, and NO_3 , P, and NH_4 were consistently higher. This would indicate that Mg, Ca, and DOC could be expected to be reliable indicators of disturbance to the soil core systems.

Variability alone is not an adequate indicator. A chemical species could be a consistent, invariate parameter; indeed, so invariate that no effect of a perturbation could be detected at all. Therefore, the analysis for variability must be supplemented with analysis of

Table 4.23. Comparison of variability among elements leached from terrestrial microcosms. Values are coefficients of variation (S.D. x 100/mean) for control groups. "R" indicates variability among replicates (C.V. calculated for each leaching date and then averaged). "T" indicates variability through time (C.V. calculated for each core and then averaged).^a

		Mg	Ca	DOC	K	NO ₃	P	NH ₄
Grassland I	R	<u>53</u>	63	64	61	79*	89*	
	T	<u>52</u>	<u>68</u>	80	<u>79</u>	151*	165*	
Grassland II	R	<u>53</u>	58	<u>54</u>	69	123*	85*	
	T	<u>62</u>	83	<u>57</u>	68	140*	114*	
Treecosc I	R	20	23		46	140*	61*	
	T	<u>53</u>	<u>43</u>		126*	69*	109*	
Treecosc II	R		50	35	55	114*	70*	63
	T		<u>43</u>	<u>63</u>	84	148*	95	159*
Washburn	R	28	27	34	55	57*	63*	
	T	<u>54</u>	<u>54</u>	93	69	102*	98*	
O'Neill (intact)	R	40	42	37	47	87*	81	91*
	T	<u>43</u>	<u>44</u>	<u>51</u>	38	112	173*	115*
Dosage Cd	R		56	43		64*		
	T		<u>36</u>	<u>73</u> *		<u>70</u>		

^aThe two lowest coefficients are underlined in each comparison and the two largest are indicated by asterisk.

sensitivity. In one approach we performed an analysis of variance on all the experiments. Table 4.24 gives the alpha values for the significance of a treatment effect when the experiments are analyzed for each element separately. By examining the alpha values which are less than 0.01, it can be seen that Mg and Ca show reasonable levels of sensitivity, probably due to the low variability of these elements. The DOC appears less reliable than when variability alone is considered, being quite insensitive in four of the experiments. Phosphorus and NH_4 continue to show themselves as less than useful. The most significant new information gained from this analysis is that NO_3 now appears as a sensitive indicator. That is, in spite of the variability of NO_3 data, the response to perturbation was large enough to clearly indicate a response.

Table 4.25 shows a different indicator of sensitivity. In this case the three pretreatment leachates were averaged and compared to the three posttreatment leachates. A measure of sensitivity would then be the difference in these means divided by the variances, i.e., a calculated t value, which is presented in the table. It should be emphasized that this is not proposed as a test of significance, which is the usual connotation within which the t value is calculated. Here we are simply interested in the sensitivity of the chemical species. Therefore, the difference between pre- and posttreatment mean values, weighted by the total variance, is a reasonable representation of sensitivity. It is incidental that this measure is equal to a calculated t.

Table 4.25 confirms the impressions of sensitivity which were gained from Table 4.24. Magnesium and Ca continue to appear as

Table 4.24. Alpha values for the significance of treatment (toxic substance) effect in eight experiments. Results are shown for total element export (not concentrations).^a

	Mg	Ca	DOC	K	NO ₃	P	NH ₄
Grassland I	NS ^b	NS	NS	0.0372	<u>0.0026</u>	NS	
Grassland II	0.0022	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	<u>0.0001</u>	
Van Voris	<u>0.0005</u>	<u>0.0001</u>	0.0159	NS	0.0115	NS	NS
Treecosc I	<u>0.0005</u>	0.0520		<u>0.0010</u>	<u>0.0033</u>	NS	
Treecosc II		0.0315	<u>0.0001</u>	NS	<u>0.0001</u>	NS	NS
Washburn	0.0308	0.0237	NS	NS	<u>0.0006</u>	<u>0.0002</u>	
O'Neill		0.0114	NS	0.0220	NS	NS	NS
Cd Dosage		<u>0.0001</u>	NS		<u>0.0001</u>		

^aUnderlined values are significant.

^bEffects that are not significant at the 0.05 level are indicated by NS.

Table 4.25. Average "t" values compared among elements. Values are based on three pretreatment leaching samples compared with three posttreatment leaching samples. A value was calculated for each replicate and averaged across treatments, substrates, location, etc. The calculated "t" values do not represent any test of significance but represent an index of sensitivity to perturbation.^a

	Mg	Ca	DOC	K	NO ₃	P	NH ₄
Grassland I	<u>1.087</u>	0.63	0.14*	0.44	<u>1.81</u>	0.38*	
Grassland II	<u>2.33</u>	<u>1.88</u>	0.63*	0.17*	1.12	1.38	
Van Voris	0.85	0.73*	<u>2.59</u>	0.91	1.69	0.57*	<u>1.74</u>
Treecosc II		1.08	0.30*	1.55	<u>2.72</u>	<u>2.64</u>	0.22*
O'Neill		<u>1.71</u>	0.23*	1.24	<u>1.92</u>	0.83	0.71*
Dosage Cd		1.67	<u>2.62</u>		0.89*		

^aThe two largest coefficients are underlined and the two lowest are indicated by an asterisk.

reliable and sensitive indicators. The DOC again shows itself as unreliable in many experiments, and NO_3 again appears as a sensitive indicator. Phosphorus and NH_4 show little to recommend them.

In all of the tables, K appears in an intermediate position. It is neither the most variable nor the least. It is neither the most sensitive nor the least. Thus, while there is little evidence to eliminate K, measurement of this element would certainly be given lower priority.

On the basis of the variability and sensitivity tests, the following conclusions can be reached. Although NO_3 is highly variable, it consistently shows itself to be a sensitive indicator and should be definitely included in screening programs. Magnesium and Ca are extremely reliable indicators and show sufficient sensitivity to be useful in screening programs. Phosphorus and NH_4 can be eliminated as neither reliable nor sensitive. The DOC appears consistent but relatively insensitive and cannot be given high priority in any screening application. Potassium retains an intermediate, low priority position.

One further piece of information can be put forward in the attempt to reduce the number of chemical species to be measured. Table 4.26 shows that the behavior of Mg and Ca are highly correlated in all the experiments in which they were both measured. As should be intuitively obvious from the nature of these cations, it would be sufficient to measure one or the other in any screening application.

In summary, it would appear that NO_3 plus either Ca or Mg would serve as a practical pair of chemical species to be recommended for a

Table 4.26. Correlation coefficients for Ca and Mg export.
Analysis was based on total export data.

Grassland I	0.87
Grassland II	0.99
Van Voris	0.97
Treecosc I	0.78
Washburn	0.83

screening program. Either one or the other, or both would have shown a significant treatment effect at the 0.05 alpha level (see Table 4.24) in all the experiments performed. Therefore, the synthesis of the experiments indicates that reduction of the monitoring program to these two measurements would significantly reduce the cost of a screening program, yet it would introduce minimal risk that a significant effect would be overlooked. The addition of K or DOC as another indicator might be considered, but our data indicate that they are unlikely to show an effect that would not also be detectable in NO_3 and Ca or Mg.

4.3.3 Size Effects

Examination of Table 4.21 indicates that comparisons across microcosms for the effects of size are not feasible. There are too many confounding variables to be able to select two experiments which differed in size of microcosm but in no other independent variables. Therefore, the analysis of the effect of size must be restricted to the Grassland I and Grassland II experiments, within which size was considered.

Based on nutrient variability and sensitivity, an analysis of variance was performed for Ca, Mg, NO_3 , and DOC, considering both total export and concentration. Tables 4.27 and 4.28 show means and standard deviations for these quantities aggregated across all treatments and then separated by treatment level. For noncontrol microcosms, the analysis was performed both pre- and posttreatment. Based on the calculated F-values, the size effect was significant at the $P \leq 0.01$ level for all of the comparisons indicated by an asterisk. Significant

Table 4.27. Microcosm size comparison on intact cores in Grassland I where size was a variable. Analysis of variance was performed over all treatments; controls only; lowest dose only; highest dose only. The last two computations were broken into pre- and posttreatments to see if there was a different response after As was added.^d

As dose	Nutrient	Small ^b	Large ^c		Large	Small
		X S.D.	X S.D.	X S.D.	X S.D.	X S.D.
All ^d	ExpCa ^e	1493 ± 1612	1569	1283		
	ExpNO ₃	972 ± 1369	697	1123		
	ExpDOC	7694 ± 8892	8969	15212		
	ExpMg	407 ± 477	421	492		
	Ca ^f	8.7 ± 7.1 *	14.3	15.8		
	NO ₃	4.1 ± 6.1	4.2	9.1		
	DOC	68 ± 44	79	79		
	Mg	2.9 ± 1.9	4.4	3.1		
0.0 As ^g	ExpCa	1244 ± 1287 *	2405	1948		
	ExpNO ₃	891 ± 973	1564	2309		
	ExpDOC	5873 ± 5893	9005	9857		
	ExpMg	371 ± 425	567	519		
	Ca	8.1 ± 7.6 *	26.2	37.5		
	NO ₃	5.1 ± 7.8	10.6	23.3		
	DOC	54 ± 34 *	142	160		
	Mg	2.3 ± 2.0 *	5.1	4.9		
0.05 mg As per cm ²	ExpCa	-	-	1508 ± 1657	1173 ± 891	
	ExpNO ₃	-	-	549 ± 840 *	80 ± 167	
	ExpDOC	-	-	7186 ± 6404	8166 ± 14810	
	ExpMg	-	-	420 ± 463	301 ± 272	
	Ca	6.7 ± 4.8	9.2 4.7	10.0 ± 6.3 *	6.4 ± 3.4	
	NO ₃	0.28 ± 0.37 *	0.16 0.34	3.8 ± 4.4 *	0.369 ± 0.658	
	DOC	93 ± 45	71 47	54 ± 41 *	35 ± 40	
	Mg	1.9 ± 1.5	2.2 1.4	2.8 ± 1.8 *	1.6 ± 1.1	
5.0 mg As per cm ²	ExpCa	-	-	1728 ± 1893	1129 ± 1011	
	ExpNO ₃	-	-	1476 ± 2293	446 ± 893	
	ExpDOC	-	-	10023 ± 14378	9735 ± 20970	
	ExpMg	-	-	430 ± 453	395 ± 684	
	Ca	5.7 ± 4.5	9.9 7.1	13.6 ± 12.0 *	7.6 ± 6.4	
	NO ₃	0.4 ± 0.64 *	0.11 0.21	13.1 ± 15.6 *	3.0 ± 6.5	
	DOC	84 ± 50 *	52 28	70 ± 58 *	34 ± 39	
	Mg	1.4 ± 1.3	2.4 1.8	3.3 ± 2.6	2.9 ± 5.6	

^aDifferences ($p \leq 0.01$) are indicated by an asterisk.

^b10 x 10 cm.

^c30 x 15 cm.

^dAnalysis of variance and means across all doses, pretreatment and post-treatment combined.

^eExport values = volume leachate x concentration in ppm leachate.

^fConcentration in ppm leachate.

^gControls compared, pretreatment period and posttreatment combined.

Table 4.28. Microcosm size comparison on intact cores in Grassland II. Analysis of variance was performed over all treatments; controls only; lowest dose only; highest dose only. The last two computations were broken into pre- and posttreatments to see if there was a different response after As was added.^a

As dose	Nutrient	Small ^b ($\bar{X}$ S.D.)	Large ^c ($\bar{X}$ S.D.)	Large ($\bar{X}$ S.D.)	Small ^f ($\bar{X}$ S.D.)
All ^d	ExpCa ^e	1051 ± 1259 *	5535 ± 5533		
	ExpNO ₃	130 ± 226	98 ± 107		
	ExpDOC	1696 ± 1460 *	6219 ± 4829		
	ExpMg	123 ± 84 *	629 ± 473		
	Ca ^f	3.8 ± 1.0 *	7.8 ± 4.1		
	NO ₃	0.395 ± 0.45 *	0.11 ± 0.12		
	DOC	8.9 ± 4.2 *	10.6 ± 5.7		
	Mg	0.6 ± 0.27 *	1.3 ± 0.8		
0.0 As ^g	ExpCa	1480 ± 2229	9109 ± 12582		
	ExpNO ₃	13 ± 16	25 ± 38		
	ExpDOC	1287 ± 996	1965 ± 2543		
	ExpMg	90 ± 53	633 ± 2543		
	Ca	4.96 ± 3.99	11.6 ± 5.8		
	NO ₃	0.086 ± 0.08	0.05 ± 0.08		
	DOC	6.87 ± 3.0	7.6 ± 3.0		
	Mg	0.6 ± 0.38	1.9 ± 1.5		
0.05 mg As per cm ²	ExpCa	710 ± 643 *	1755 ± 1919	809 ± 866 *	3277 ± 3855
	ExpNO ₃	13 ± 15	20 ± 34	73 ± 74	77 ± 119
	ExpDOC	1423 ± 1042 *	3420 ± 2922	1714 ± 1146 *	5775 ± 4352
	ExpMg	147 ± 138 *	412 ± 407	100 ± 51 *	348 ± 185
	Ca	3.9 ± 0.59	4.5 ± 0.8	2.2 ± 1.0 *	3.3 ± 1.2
	NO ₃	0.06 ± 0.12 *	0.12 ± 0.08	0.23 ± 0.20 *	0.12 ± 0.20
	DOC	4.0 ± 6.5	10.2 ± 4.7	5.7 ± 2.8 *	7.8 ± 5.1
	Mg	0.8 ± 0.2 *	1.2 ± 0.4	0.9 ± 0.1 *	0.6 ± 0.2
5.0 mg As per cm ²	ExpCa	712 ± 538	2862 ± 4536	1112 ± 1049 *	7096 ± 4775
	ExpNO ₃	17 ± 21	19 ± 40	650 ± 1215	424 ± 371
	ExpDOC	1434 ± 872	2403 ± 2355	4567 ± 3711 *	21784 ± 14259
	ExpMg	142 ± 86	294 ± 339	168 ± 122 *	1451 ± 1075
	Ca	3.1 ± 0.9 *	10.1 ± 9.1	3.1 ± 1.3 *	5.4 ± 1.8
	NO ₃	0.12 ± 0.14 *	0.05 ± 0.08	1.7 ± 2.1 *	0.36 ± 0.24
	DOC	8.45 ± 2.28	9.4 ± 3.8	14.6 ± 7.7 *	21.1 ± 0.2
	Mg	0.8 ± 0.4	1.1 ± 0.6	0.5 ± 14.5 *	1.08 ± 0.31

^aSignificant differences ($p \leq 0.01$) are indicated by an asterisk.

^b15 x 25 cm.

^c30 x 25 cm.

^dAnalysis of variance and means across all doses, pretreatment and post-treatment combined.

^eExport values = volume leachate x concentration in ppm leachate.

^fConcentration in ppm leachate.

^gControls compared, pretreatment period and posttreatment combined.

size effects could be detected at each treatment level and in both pre- and posttreatment samples. With few exceptions (see control dosage, Ca, DOC, and Mg concentrations) the large microcosms showed greater concentrations of leachates.

The microcosms were also compared to determine if the larger size was consistently less variable. If this were the case, one might wish to choose the larger size because smaller sample sizes would be required to detect significant differences. Table 4.29 shows coefficients of variation for Ca and NO_3 compared between large and small microcosms. No unambiguous pattern can be detected; indeed, in many cases, the larger microcosms showed greater variability both through time and between replicates.

Size effects also appeared to play a significant role in CO_2 measurements. Table 4.30 indicates that CO_2 measured from large microcosms showed smaller coefficients of variation with great consistency. This would indicate that the larger microcosms would be more reliable for this parameter.

The most important test for the significance of the size effect must be whether the larger size microcosms were more sensitive in detecting dosage effects. Table 4.31 compares the large and small microcosms in the Grassland I and II experiments. The value shown in the table is the calculated F value for treatment effect. The significant F values at $P \leq 0.01$ are indicated by an asterisk. This comparison shows that the larger size microcosms showed a clearer treatment effect and can therefore be judged as more sensitive for determining an effect. The larger the F value, the larger the ratio of treatment sum of squares

Table 4.29. Comparison of variability of Ca and NO₃ between small and large microcosms. Values are coefficients of variation (S.D. x 100/mean) for pre- and posttreatment of control, 0.5 mg As per cm², and 5.0 mg As per cm². Coefficients of variation are then tabulated among replicates (C.V. calculated for each leaching date and then averaged) and through time (C.V. calculated for each core and then averaged). C.V. between replicates is indicated by "R" and C.V. through time by "T."^a

			Ca	NO ₃
Grassland I				
Pretreatment				
Control	small ^b		78	194
	large ^c		149	191
0.5 mg As/cm ²	small		71	133
	large		51	210
5.0 mg As/cm ²	small		78	141
	large		77	189
Post				
Control	small		101	149
	large		137	219
0.5 mg As/cm ²	small		63	116
	large		54	178
5.0 mg As/cm ²	small		88	119
	large		84	220
Grassland II				
Pretreatment				
Control	small ^d		25	91
	large ^e		25	129
0.5 mg As/cm ²	small		15	92
	large		20	141
5.0 mg As/cm ²	small		24	114
	large		90	168
Post				
Control	small		110	107
	large		56	186
0.5 mg As/cm ²	small		45	88
	large		37	169
5.0 mg As/cm ²	small		43	122
	large		32	69
Grassland I	R	small	58	96
		large	92	140
	T	small	81	141
		large	59	187
Grassland II	R	small	43	107
		large	71	100
	T	small	61	104
		large	61	144

^aHigher coefficients are underlined.

^bSmall = 10 x 10 cm.

^cLarge = 30 x 15 cm.

^dSmall = 15 x 25 cm.

^eLarge = 30 x 25 cm.

Table 4.30. Comparison of CO₂ efflux of small (10x10 cm) and large (30x15 cm) intact core microcosms. Coefficient of variation (S.D. x 100/mean) were calculated after treatment for all treatments combined, controls only, 0.5 mg As/cm²; 5.0 mg As/cm². Values among replicates (C.V. calculated for each leaching date and then averaged) are designated by "R" and values through time (C.V. calculated for each core and then averaged) are designated by "T."^a

<u>Grassland I</u>		
	Size	C.V.
All ^b	Small ^c	35
	Large ^d	<u>13</u>
Controls ^e	Small	26
	Large	<u>15</u>
0.5 mg/cm ²	Small	45
	Large	<u>14</u>
5.0 mg/cm ²	Small	34
	Large	<u>11</u>
Grassland I		
R	Small	57
	Large	<u>48</u>
T	Small	39
	Large	<u>15</u>

^aHighest coefficients are underlined.

^bC.V. averaged over all microcosms.

^c10 x 10 cm.

^d30 x 15 cm.

^eNo arsenic added.

Table 4.31. Calculated F values for treatment effect in two microcosm experiments. The significant values at $P \leq 0.01$ are indicated by an asterisk. The values show that the larger microcosms were able to detect a clearer treatment effect.

		Large	Small
Grassland I	Ca	11.5*	0.5
	NO ₃	4.5*	3.2
	ExpCa	7.6*	0.6
	ExpNO ₃	7.9*	2.6
Grassland II	Ca	12.8*	4.0
	NO ₃	8.7*	8.4*
	ExpCa	3.5	1.7
	ExpNO ₃	11.3*	4.2

to error sum of squares; therefore, the larger F value indicates that, compared to the residual error, more of the variance in the large microcosm measurements could be attributed to a dosage effect. This result can be interpreted to mean that the large cores are more sensitive to the heavy metal treatment, or that at any given treatment level fewer samples of the larger size would be required to detect a significant effect.

However, the greater sensitivity of the larger microcosms does not unambiguously answer the question of whether the larger sizes should be used in screening programs. Table 4.24 shows very clearly that treatment effects could be detected in all of our experiments in either Ca or NO₃ leachate. It is clear, therefore, that smaller size microcosms have sufficient sensitivity to detect an effect. The question of small versus large microcosm size must be answered, based on the practicality and expense of the screening operation. The answer would therefore involve greater experience with the problems of large-scale screening programs and the economic balance of using a few large microcosms versus a larger number of small cores. This question seems best answered as more experience is gained with microcosms as screening tools.

4.3.4 Substrate Effects

The significant effects of microcosm substrate have been shown in several of our studies. Jackson et al. (in press) showed that forest soils were more sensitive to arsenic disturbance, particularly evident in greater losses of calcium compared to grassland soils. O'Neill et al. (unpublished manuscript) showed that there were significant

differences between an artificial sand-soil substrate and either intact soil cores or homogenized soil cores. There were only small differences between the intact and homogenized microcosms, and it was argued that the intact substrate should be chosen because it retained a more natural array of interacting biological populations. The additional work involved in preparing the homogenized substrate did not appear to be justified. Because much of the evidence for substrate effect has already been presented in manuscripts generated from individual experiments, little remains except to complete the analysis by presenting evidence from the Grassland II experiment which has not yet reached the literature.

Analysis of variance of the Grassland II experiment indicates that there was only a single significant difference ($P \leq 0.01$) among substrate types (i.e., intact versus homogenized). Table 4.32 presents means and standard deviations for total export and concentration of Ca and NO_3 . At the highest dose level, the intact cores lost significantly more nitrates. None of the other measurements showed significant differences. The scarcity of significant differences is in keeping with the findings of O'Neill et al. (unpublished manuscript). The argument would again seem to hold that because the intact cores require less preparation time and, are less expensive, they should be the substrate of choice for large-scale screening programs.

Table 4.33 compares the variability of the intact and homogenized cores for Grassland II. The major argument for using homogenized soil substrates is that homogenization tends to decrease variability. This decreased variability for the homogenized substrate might be arguable

Table 4.32. Homogenized grassland soil cores and intact grassland cores were compared in Grassland II. Analysis of variance was performed over all treatments; controls only; lowest dose only; highest dose only. The last two computations were broken into pre- and posttreatments to see if there was a different response after As was added.

As dose	Nutrient	Pretreatment		Posttreatment	
		Intact ($\bar{X} \pm S.D.$)	Homogenized ($\bar{X} \pm S.D.$)	Intact ($\bar{X} \pm S.D.$)	Homogenized ($\bar{X} \pm S.D.$)
All ^a	ExpCa ³	5535 $\pm$ 5533	7098 $\pm$ 28317		
	ExpNO ₃	98 $\pm$ 107	395 $\pm$ 570		
	Ca ^c	7.8 $\pm$ 4.1	8.1 $\pm$ 5.9		
	NO ₃	0.12 $\pm$ 0.13	0.41 $\pm$ 0.42		
Controls ^d	ExpCa	9109 $\pm$ 12582	16281 $\pm$ 24310		
	ExpNO ₃	25 $\pm$ 38	20 $\pm$ 39		
	Ca	11.6 $\pm$ 5.8	15.7 $\pm$ 13.7		
	NO ₃	0.05 $\pm$ 0.08	0.06 $\pm$ 0.06		
0.5 mg As per cm ²	ExpCa	1755 $\pm$ 1919	666 $\pm$ 419	3277 $\pm$ 3855	3935 $\pm$ 5124
	ExpNO ₃	20 $\pm$ 34	17 $\pm$ 21	77 $\pm$ 119	230 $\pm$ 562
	Ca	4.4 $\pm$ 0.8	3.7 $\pm$ 0.7	3.3 $\pm$ 1.2	3.8 $\pm$ 2.0
	NO ₃	0.12 $\pm$ 0.07	0.1 $\pm$ 0.1	0.12 $\pm$ 0.20	0.13 $\pm$ 0.20
5.0 mg As per cm ²	ExpCa	2862 $\pm$ 4536	938 $\pm$ 1112	7096 $\pm$ 4775	4487 $\pm$ 4556
	ExpNO ₃	19 $\pm$ 40	6 $\pm$ 5	424 $\pm$ 371	2064 $\pm$ 2751
	Ca	10.2 $\pm$ 9.1	4.4 $\pm$ 1.4	0.36 $\pm$ 0.239	5.2 $\pm$ 3.8
	NO ₃	0.05 $\pm$ 0.08	0.08 $\pm$ 0.09	21.1 $\pm$ 0.20	5.2 $\pm$ 2.0

^aAnalysis of variance and means across all doses, pretreatment and post-treatment combined.

^bExport values = volume leachate x concentration in ppm leachate.

^cConcentration in ppm leachate.

^dNo As added, pretreatment and posttreatment combined.

Table 4.33. Comparison of variability of Ca and NO₃ between intact and homogenized microcosms. Values are coefficients of variation (S.D. x 100/mean) for pre- and posttreatment of control 0.5 mg As per cm², and 5.0 mg As per cm². Coefficients of variation are then tabulated among replicates (C.V. calculated for each leaching date and then averaged) and through time (C.V. calculated for each core and then averaged). C.V. between replicates is indicated by "R" and C.V. through time by "T."^a

			Ca	NO ₃
Grassland II				
Pretreatment				
Control	Intact		25	129
	Homogenized		<u>11</u>	<u>92</u>
0.5 mg As/cm ²	Intact		20	141
	Homogenized		<u>18</u>	<u>107</u>
5.0 mg As/cm ²	Intact		90	168
	Homogenized		<u>32</u>	<u>110</u>
Posttreatment				
Control	Intact		56	186
	Homogenized		97	<u>126</u>
0.5 mg As/cm ²	Intact		<u>37</u>	169
	Homogenized		<u>52</u>	<u>152</u>
5.0 mg As/cm ²	Intact		<u>32</u>	69
	Homogenized		<u>73</u>	<u>93</u>
Grassland II	R	Intact	71	100
		Homogenized	<u>55</u>	100
	T	Intact	61	144
		Homogenized	<u>75</u>	<u>130</u>

^aHigher coefficients are underlined.

from the coefficients of variation for pretreatment codes in Table 4.33. The intact cores consistently had higher coefficients for both Ca and NO_3 . However, examination of the posttreatment data indicates that the pattern is not consistent. For NO_3 , the intact core was more variable for two out of three dose levels. For Ca, the homogenized cores were more variable in every case. Lumping across all the data (bottom of Table 4.33) fails to show either the intact or the homogenized substrate as consistently less variable.

Because the major argument for using the homogenized substrate is the potential for decreased variability, and because the data from our experiments indicate that there is not a consistent trend for lower variability in the homogenized cores, there seems little argument for the homogenized substrate. In the small cores suggested for large-scale screening of toxic substances, the homogenized cores are actually more expensive to produce. Therefore, small intact cores should be the recommended basis for the screening programs.

4.4 SIMULATION MODELING AND APPLICATION TO MICROCOSM AND FIELD DATA (R. J. Luxmoore)

Understanding of the chemical, physical, and physiological processes involved in chemical transport in terrestrial ecosystems can be aided with simulation models based on these processes. The complexity of process interactions within plants and the litter system, however, may require that the phenomenological behavior of chemical transport be modeled with simplified empirically derived functions. Nevertheless, the continuing aim of process modeling is to represent the principles of chemistry, physics, and physiology. One attempt at modeling the

carbon, water, and chemical fluxes and pools in the soil-plant-litter system was made in the development of the Unified Transport Model (Baes et al. 1976). Five models were developed and coupled together and executed with hourly resolution of photosynthesis, translocation, respiration, transpiration, solute and water uptake, litter decomposition, and chemical mineralization (Table 4.34). The sensitivity of the model output to changes in input parameters is critical information in model applications because it defines the precision with which input data should be obtained for a given precision of output results. Sensitivity analysis also serves to identify important parameters and important functions (from the model's viewpoint) that may aid in evaluation of real-world phenomena.

Evaluation of the environmental hazard from chemical releases into terrestrial ecosystems often needs to be made by regulatory agencies without full information about the nature of the chemical-environment-biota interactions. Modeling can facilitate the evaluation process at the quantitative, semiquantitative, or qualitative levels of resolution. The first part of this study was conducted as an aid in evaluation of chemical transport in the soil-plant-litter system by providing sensitivity information about model parameters and seeking the identification of critical steps in chemical transport in which water, carbon, and chemicals are represented as coupled components.

Several experimental techniques are available for assessing the short-term effects of chemical releases into the environment. These testing protocols include the use of basic chemical characterization (volatility, solubility, pH), animal and plant toxicity (LD_{50}), and

Table 4.34. Some attributes of coupled models describing carbon, water, and chemical dynamics in the soil plant-litter system

Component model	Soil-plant-atmosphere water flow	Soil chemistry	Vegetation and litter carbon dynamics	Diffusion and mass flow of solutes	Solute dynamics in vegetation and litter
Name	PROSPER (TEHM)	SCHEM	CERES	DIFMAS	DRYADS
Time step	15 or 60 min	15 or 60 min	60 min	15 or 60 min	15 or 60 min
Characteristics	Evapotranspiration by combination method. Soil water flow by Darcy flow equation. Uses empirical relationship between surface resistance and surface water potential. Empirical data for soil hydraulic properties.	Uses empirical distribution coefficient (K_d) for soil of interest.	CO ₂ diffusion equation for net photosynthesis. Substrate gradient equation for translocation. Uses input values for potential growth of plant components. Empirical litter decomposition relationships.	Implements model of diffusion and mass flow of solutes to roots by Baldwin et al. (1973). Transpiration flux used for xylem transport. Plant demand function determined by potential solute concentration input values.	Solute uptake by roots and leaves. Diffusive gas uptake by leaves. Gradient equation for phloem translocation.
Documentation Report number	ORNL/NSF/EATC-27	ORNL/NSF/EATC-16	ORNL/NSF/EATC-25	ORNL/NSF/EATC-26	ORNL/NSF/EATC-26
Authors	Huff et al. (1977)	Begovich and Jackson (1975)	Dixon et al. (1976, 1978a,b)	Luxmoore et al. (1976, 1978a)	Luxmoore et al. (1976, 1978a)

soil and sediment adsorption. Chemical movement, accumulation, and effects in terrestrial and aquatic microcosms can also give short-term evaluations (months) of possible hazards from chemical releases into the environment. It is not clear, however, that short-term evaluations can reveal longer-term hazards to an ecosystem with its complicated component interactions, turnover rates, accumulations, and seasonality. Thus it is possible that a chemical may be viewed as "safe" according to testing protocols but prove to be hazardous at some longer time after release. It is not always feasible to conduct long-term (several years) ecosystem testing of new chemicals. Simulation models based on physical, chemical, and physiological principles offer one means of looking into the future and may provide valuable assistance in testing protocols as the models gain in sophistication and their input data gain in reliability.

The second part of this study evaluates the efficacy of using microcosm data to calibrate models for the prediction of long-term chemical transport in terrestrial ecosystems.

4.4.1 Parameter Sensitivity Studies

4.4.1.1 Materials and Methods

The parameters selected for investigation (Table 4.35) represent a range of soil and plant properties that may influence chemical transport. The primary model where the parameter is used is given along with a description and the range of values used in the analysis. The soil, vegetation, litter, and climatic data set used in the Crooked Creek Watershed simulation (Munro et al. 1976) were chosen as the

Table 4.35. Standard parameter values and tested values for sensitivity analyses

	Variable name	Standard values	Tested values ^a
Soil and chemical parameters (SCEHM)			
Distribution coefficient (ml/g) for upper and lower soil layers	K _d	500, 600 (lead) 10, 10 (zinc)	2500, 3000 100, 125 (lead) 1000, 1000 100, 100 (zinc) 1, 1
Solubility (μg/ml)	SP	3 (lead) 2 (zinc)	300, 30 (lead) 200, 20 (zinc)
Diffusion coefficient (cm ² /sec)	DL	10 ⁻⁵	10 ⁻⁷ , 10 ⁻³ , 10 ⁻¹
Litter parameter (CERES)			
Decomposition rate constant (g g ⁻¹ hr ⁻¹)	DMAX	3.5 x 10 ⁻⁴ (fruit) 5.0 x 10 ⁻⁵ (leaf) 1.8 x 10 ⁻⁴ (stem) 1.3 x 10 ⁻⁴ (root)	x 10, x 10 ⁻¹ x 10, x 10 ⁻¹ x 10, x 10 ⁻¹ x 10, x 10 ⁻¹
Plant parameters (CERES)			
Phloem resistances (hr)			
Leaf to stem	LSPHLO	20	100, 1
Stem to root	SRPHLO	30	100, 1
Stem to fruit	SFPHLO	500	1000, 100
Respiration rates (g g ⁻¹ hr ⁻¹)			
Root	RRESTD	1.8 x 10 ⁻⁴	x 10, x 10 ⁻¹
Stem	SRESTD	1.9 x 10 ⁻⁴	x 10, x 10 ⁻¹
Leaf	LRESTD	1.8 x 10 ⁻⁴	x 10, x 10 ⁻¹
Fruit	FRESTD	1 x 10 ⁻⁴	x 10, x 10 ⁻¹
Leaf area to weight ratio (cm ² /g)	ARI	100	200, 50
Water potential effects (bars)	EI	4	-2, +2
Root radius (cm)	R	0.05	5, 0.5, 0.005
Maximum leaf storage (g/m ²)	L MAX	420	210, 42
Ambient CO ₂ concentration (ml/ml)	CO2X	3.2 x 10 ⁻⁴	x2, x3, x4
Plant uptake parameters (DRYADS)			
Leaf cuticle permeability (cm/sec)	PERM	10 ⁻¹¹	10 ⁻⁷ , 10 ⁻⁹ 10 ⁻¹⁰ , 10 ⁻¹³
Cuticle thickness (cm)	FILM	10 ⁻⁴	10 ⁻² , 10 ⁻⁵ , 10 ⁻⁶
Root solute conductivity (cm/sec)	CONDOC	10 ⁻⁸	10 ⁻⁶ , 10 ⁻¹⁰ , 10 ⁻¹²
External SO ₂ concentration (ml/ml)	GASEX	2 x 10 ⁻⁸	2 x 10 ⁻⁷ , 2 x 10 ⁻⁹

^axn or +n implies that the standard value was multiplied by n or added to n, respectively.

standard set of input values. Each parameter was varied around this standard, and the results were related back to this standard set of results. The output parameter list used in the analysis (Table 4.36) is extensive and provides many points at which the system sensitivity may be evaluated.

The method for testing the parameters consisted of running the model over a three-month period, May-July, with one particular value varied in the standard set of input. No data were recorded for the initial two-month period of the simulations to allow the model to stabilize. Daily values were output on file for the entire month of July, and hourly values were recorded on file for a period of eight days (16th-24th) during July for sensitivity and statistical analysis.

Two indices of variation were computed for each response over time for all sensitivity runs. The first, termed a relative output coefficient (r), measures the output response with respect to the standard response and has similarities with the χ^2 statistical test.

$$r_j^{(k)} = \frac{\sum_{i=1}^T \left(\frac{a_{ij}^{(k)} - e_{ij}}{e_{ij}} \right)^2}{T},$$

where e_{ij} = response of the j -th output parameter for i -th time step in the standard run,

a_{ij} = response of the j -th output parameter for the i -th time step in sensitivity run k ,

T = total number of time steps, and

r_j = relative output coefficient for the j -th output parameter in the k -th sensitivity run.

Table 4.36. Model output variables considered in this study

EXCHANGEABLE LEAD (UG/G) IN 0-3 CM SOIL LAYER	EXCHANGEABLE LEAD (UG/G) IN 3-15 CM SOIL LAYER
EXCHANGEABLE ZINC (UG/G) IN 0-3 CM SOIL LAYER	EXCHANGEABLE ZINC (UG/G) IN 3-15 CM SOIL LAYER
EXCHANGEABLE ZINC (UG/G) IN 15-30 CM SOIL LAYER	EXCHANGEABLE ZINC (UG/G) IN 15-30 CM SOIL LAYER
ROOT BIOMASS (G/M ² LAND)	LEAF BIOMASS (G/M ² LAND)
STEM BIOMASS (G/M ² LAND)	STEM BIOMASS (G/M ² LAND)
ROOT RESPIRATION (G/M ² LAND/HR)	LEAF RESPIRATION (G/M ² LAND/HR)
STEM RESPIRATION (G/M ² LAND/HR)	STEM RESPIRATION (G/M ² LAND/HR)
STEM TO ROOT SUGAR TRANSLLOCATION (G/M ² LAND/HR)	LEAF TO STEM SUGAR TRANSLLOCATION (G/M ² LAND/HR)
STEM TO FRUIT SUGAR TRANSLLOCATION (G/M ² LAND/HR)	CO ₂ CHLOROPLAST (ML/ML)
PHOTOSYNTHESIS (G CO ₂ /CM ² LEAF/HR)	LEAF LEAF CONCENTRATION (UG/G)
LEAF ZINC CONCENTRATION (UG/G)	LEAF SULFUR CONCENTRATION (UG/G)
FRUIT LEAD CONCENTRATION (UG/G)	FRUIT ZINC CONCENTRATION (UG/G)
FRUIT SULFUR CONCENTRATION (UG/G)	STEM LEAF CONCENTRATION (UG/G)
STEM ZINC CONCENTRATION (UG/G)	STEM SULFUR CONCENTRATION (UG/G)
STEM HEARTWOOD LEAD CONCENTRATION (UG/G)	STEM HEARTWOOD ZINC CONCENTRATION (UG/G)
ROOT ZINC CONCENTRATION (UG/G)	ROOT LEAD CONCENTRATION (UG/G)
ROOT HEARTWOOD LEAD CONCENTRATION (UG/G)	ROOT SULFUR CONCENTRATION (UG/G)
ROOT HEARTWOOD SULFUR CONCENTRATION (UG/G)	ROOT HEARTWOOD ZINC CONCENTRATION (UG/G)
ZINC IN STEM LITTER (UG/M ² LAND)	LEAD IN STEM LITTER (UG/M ² LAND)
SULFUR IN FRUIT LITTER (UG/M ² LAND)	SULFUR IN STEM LITTER (UG/M ² LAND)
ZINC IN ROOT LITTER (UG/M ² LAND)	ZINC IN FRUIT LITTER (UG/M ² LAND)
LEAD CONTENT IN LEAF XYLEM (UG/M ² LAND)	SULFUR IN ROOT LITTER (UG/M ² LAND)
SULFUR CONTENT IN LEAF XYLEM (UG/M ² LAND)	ZINC CONTENT IN LEAF XYLEM (UG/M ² LAND)
ZINC CONTENT IN STEM XYLEM (UG/M ² LAND)	SULFUR CONTENT IN STEM XYLEM (UG/M ² LAND)
LEAD CONTENT IN ROOT XYLEM (UG/M ² LAND)	ZINC CONTENT IN ROOT XYLEM (UG/M ² LAND)
SULFUR CONTENT IN ROOT XYLEM (UG/M ² LAND)	LEAD MINERALIZATION FROM LEAF LITTER (UG/M ² /DAY)
ZINC MINERALIZATION FROM LEAF LITTER (UG/M ² /DAY)	ZINC MINERALIZATION FROM LEAF LITTER (UG/M ² /DAY)
SULFUR MINERALIZATION FROM STEM LITTER (UG/M ² /DAY)	LEAD MINERALIZATION FROM STEM LITTER (UG/M ² /DAY)
ZINC MINERALIZATION FROM STEM LITTER (UG/M ² /DAY)	SULFUR MINERALIZATION FROM FRUIT LITTER (UG/M ² /DAY)
LEAD MINERALIZATION FROM FRUIT LITTER (UG/M ² /DAY)	SULFUR MINERALIZATION FROM FRUIT LITTER (UG/M ² /DAY)
SULFUR MINERALIZATION FROM ROOT LITTER (UG/M ² /DAY)	ZINC MINERALIZATION FROM ROOT LITTER (UG/M ² /DAY)
ZINC MINERALIZATION FROM ROOT LITTER (UG/M ² /DAY)	LEAD INPUT TO LEAF LITTER (UG/M ² /DAY)
LEAD INPUT TO LEAF LITTER (UG/M ² /DAY)	SULFUR INPUT TO LEAF LITTER (UG/M ² /DAY)
SULFUR INPUT TO STEM LITTER (UG/M ² /DAY)	ZINC INPUT TO STEM LITTER (UG/M ² /DAY)
ZINC INPUT TO FRUIT LITTER (UG/M ² /DAY)	LEAD INPUT TO FRUIT LITTER (UG/M ² /DAY)
SULFUR INPUT TO ROOT LITTER (UG/M ² /DAY)	SULFUR INPUT TO FRUIT LITTER (UG/M ² /DAY)
LEAD INPUT TO ROOT LITTER (UG/M ² /DAY)	ZINC INPUT TO ROOT LITTER (UG/M ² /DAY)
LEAD LEACHED FROM LEAVES (UG/M ² /DAY)	SQ ₂ UPTAKE (UG/CM ² LEAF/DAY)
LEAD UPTAKE BY LEAVES (UG/M ² /DAY)	ZINC LEACHED FROM LEAVES (UG/M ² /DAY)
LEAD TO STEM PLOEM TRANSLLOCATION OF LEAD (UG/M ² LAND/HR)	ZINC UPTAKE BY LEAVES (UG/M ² /DAY)
LEAD TO STEM PLOEM TRANSLLOCATION OF SULFUR (UG/M ² LAND/HR)	LEAD TO STEM PLOEM TRANSLLOCATION OF ZINC (UG/M ² LAND/HR)
STEM TO ROOT PLOEM TRANSLLOCATION OF ZINC (UG/M ² LAND/HR)	STEM TO ROOT PLOEM TRANSLLOCATION OF LEAD (UG/M ² LAND/HR)
STEM TO FRUIT PLOEM TRANSLLOCATION OF LEAD (UG/M ² LAND/HR)	STEM TO ROOT PLOEM TRANSLLOCATION OF SULFUR (UG/M ² LAND/HR)
STEM TO FRUIT PLOEM TRANSLLOCATION OF SULFUR (UG/M ² LAND/HR)	STEM TO FRUIT PLOEM TRANSLLOCATION OF ZINC (UG/M ² LAND/HR)
STEM TO LEAF XYLEM TRANSPORT OF ZINC (UG/M ² LAND/HR)	STEM TO LEAF XYLEM TRANSPORT OF LEAD (UG/M ² LAND/HR)
STEM TO LEAF XYLEM TRANSPORT OF LEAD (UG/M ² LAND/HR)	STEM TO LEAF XYLEM TRANSPORT OF SULFUR (UG/M ² LAND/HR)
ROOT TO STEM XYLEM TRANSPORT OF ZINC (UG/M ² LAND/HR)	STEM TO STEM XYLEM TRANSPORT OF SULFUR (UG/M ² LAND/HR)
ROOT TO STEM XYLEM TRANSPORT OF LEAD (UG/M ² LAND/HR)	ROOT UPTAKE OF LEAD - SUNLIGHT PERIOD (UG/M ² LAND/DAY)
ROOT TO STEM XYLEM TRANSPORT OF SULFUR (UG/M ² LAND/HR)	ROOT UPTAKE OF ZINC - SUNLIGHT PERIOD (UG/M ² LAND/DAY)

The second index (S) relates the change in output to the change in input and is called a sensitivity coefficient,

$$S_j^{(k)} = \sum_{i=1}^T \frac{a_{ij}^{(k)} - e_{ij}}{v_k - v_k},$$

where v_k = value of input parameter that was varied for the k-th sensitivity run,

v_k = value of standard parameter for v_k used in the standard run, and

$S_j^{(k)}$ = sensitivity coefficient for k-th sensitivity run and the j-th parameter.

Values of these coefficients for each variation of input parameters are documented by Begovich and Luxmoore (1979).

The overall effect of change in each of the parameters is presented in graphs of the heavy metal contaminant proportions in each of the soil, litter, and plant components being modeled. The data can also be plotted as the variation of one response over time for different input values. The amount of data is too extensive for all of the output to be examined in this manner and some selected plots provide insight into the responses.

4.4.1.2 Results

Soil and Chemical Parameters

Distribution coefficient (K_d). The distribution coefficient is the ratio of the amount of contaminant exchanged onto the soil surface per unit soil mass to the amount of contaminant in the soil solution

per unit solution volume. Increasing this ratio increased the amount on the soil exchange, reduced the soil solution concentration, and thus reduced the total plant uptake of lead (Fig. 4.9). A similar result was obtained for zinc. A large variation of the fraction of contaminant in the plant parts was also found. The relative contaminant fraction in the leaves increased along with a decrease in both the root and stem contaminant fraction as the K_d increased. The mineralized contaminant decreased with increase in zinc K_d , whereas there was little change with increase in lead K_d .

Hourly plots of phloem (Fig. 4.10a) and xylem (Fig. 4.10b) translocation of zinc show greater transport with lower K_d and that phloem translocation can remain at high rates at night. Both transports vary indirectly with K_d . The most sensitive outputs include the chemical content in litter and roots and in the plant xylem transport pathway.

Solubility (SP). The amount of heavy metal contaminant which dissolves in the infiltrating soil water depends on the chemical solubility (SP) and this usually changes for different chemical compounds. The fraction of zinc and lead in the soil increased with increasing solubility, causing the fraction of chemical in the litter to decrease (Fig. 4.11). The fraction in each of the separate soil layers and in the biomass was basically unchanged. The overall fraction of lead in the plant increased slightly because of increased root uptake. The fraction of zinc in the plant did not vary due to the plant having attained near maximum concentrations in each of its tissues. The increase in the litter-mineralized fraction resulted from both the decrease in total litter content and a mineralization rate proportional

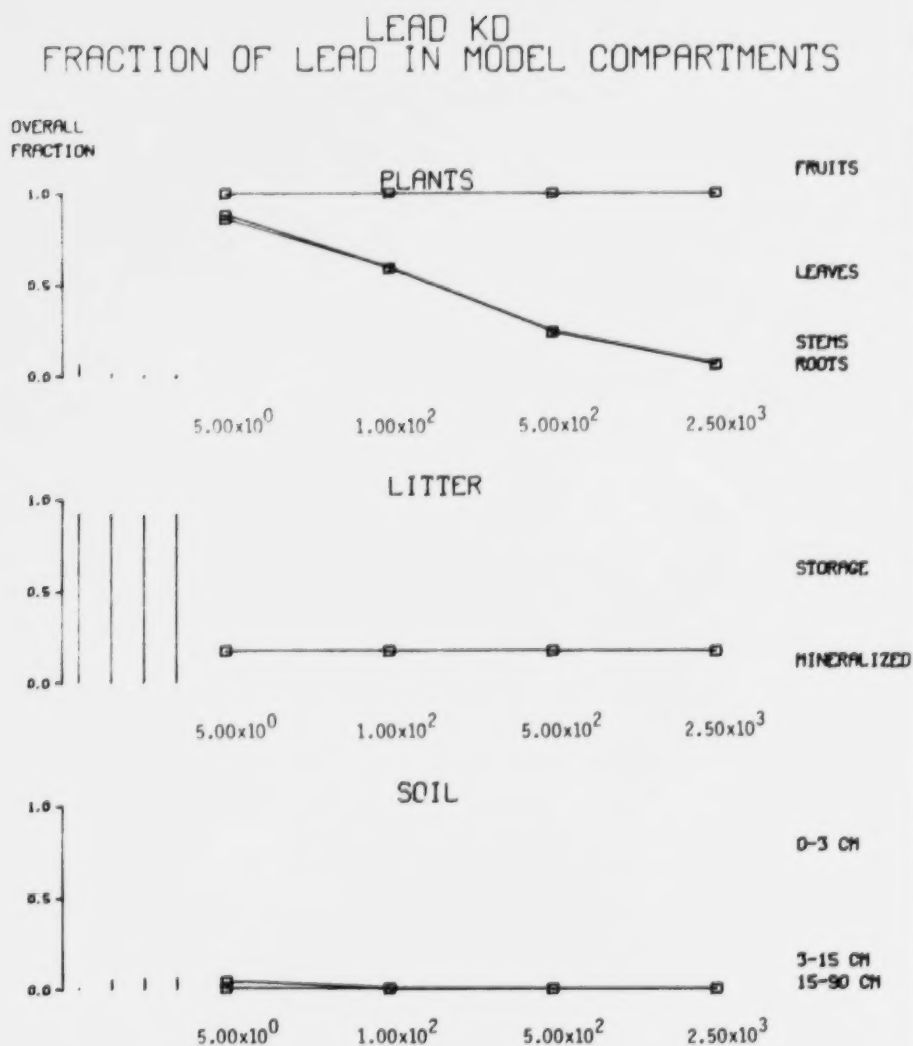


Figure 4.9. Relative lead distribution in plant, litter, and soil components with change in lead distribution coefficient (K_d).

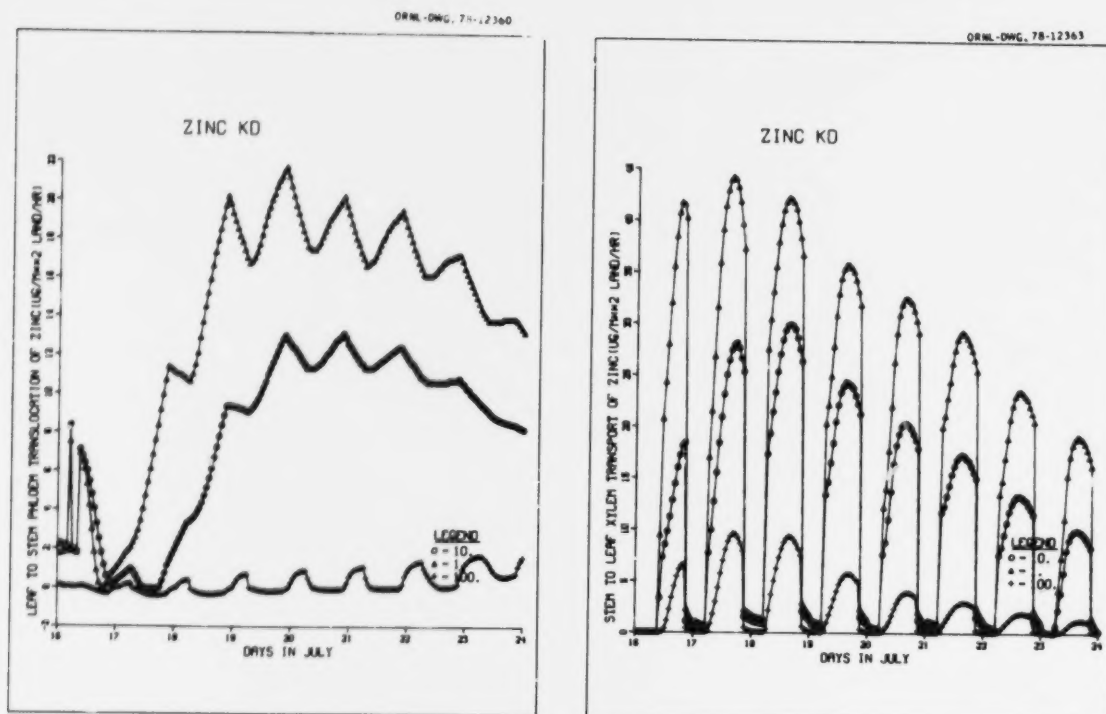


Figure 4.10. Influence of zinc K_d on zinc transport in the vegetation: (a) leaf-to-stem phloem, and (b) stem-to-leaf xylem.

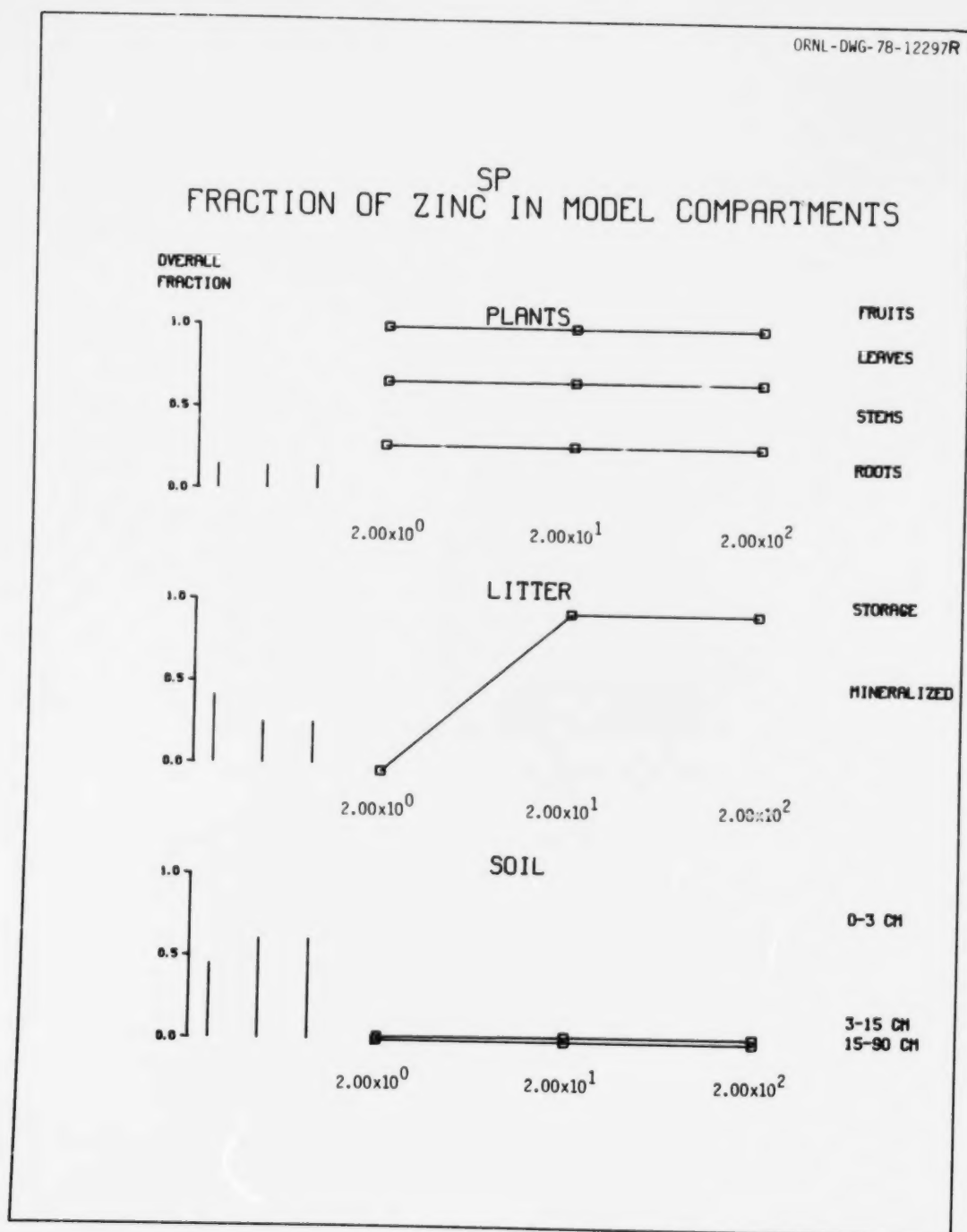


Figure 4.11. Relative zinc distribution in plant, litter, and soil components with change in zinc solubility (SP).

to litter decomposition. As for the distribution coefficient, the most sensitive outputs to changes in SP include the chemical content in litter compartments and in the plant tissue xylem.

Diffusion coefficient (DL). The diffusion coefficient of the heavy metals is used in determining root uptake. Diffusive uptake increases for increasing values of $\alpha a/(DL)b$, where α is the root absorbing power, a is the root radius, DL is the diffusion coefficient, and b is the buffer power (Baldwin et al. 1973). Thus as DL increases, diffusive uptake decreases. The overall fraction of contaminant in the plant decreased for increasing diffusion coefficient for lead (Fig. 4.12), but was unchanged for zinc because the vegetation attained a maximum internal zinc concentration. This explained the differing rates of chemical uptake for zinc and lead. Hourly root uptake during the day showed greater rates for lead with decrease in DL whereas zinc uptake was a reverse response. However, maximum root uptake for zinc occurred at the lowest value of DL . Zinc content in litter and plant xylem tissues showed the greatest sensitivity to change in DL . The most sensitive response for lead was in the lead concentration of roots.

Litter Parameter

Decomposition rate constant (DMAX). The rate of material decomposed in each of the litter compartments is a function of temperature and an input value for the decomposition rate constant. Chemical mineralization from litter is proportional to the decomposition rate. Thus, increase in the rate constant increased the amount of lead and zinc mineralization (Fig. 4.13). There was no change in overall

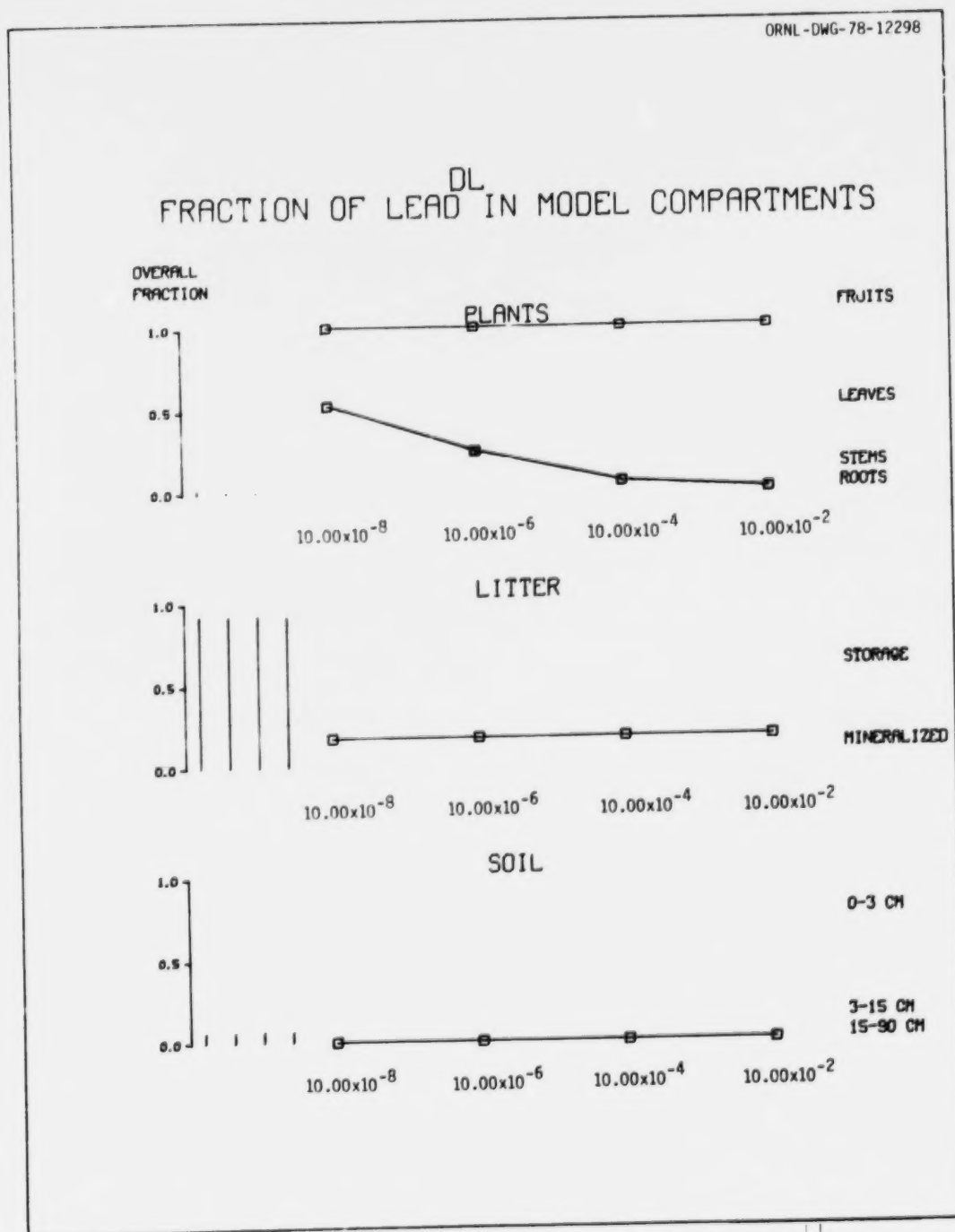


Figure 4.12. Relative lead distribution in plant, litter, and soil components with change in diffusion coefficient of lead (DL).

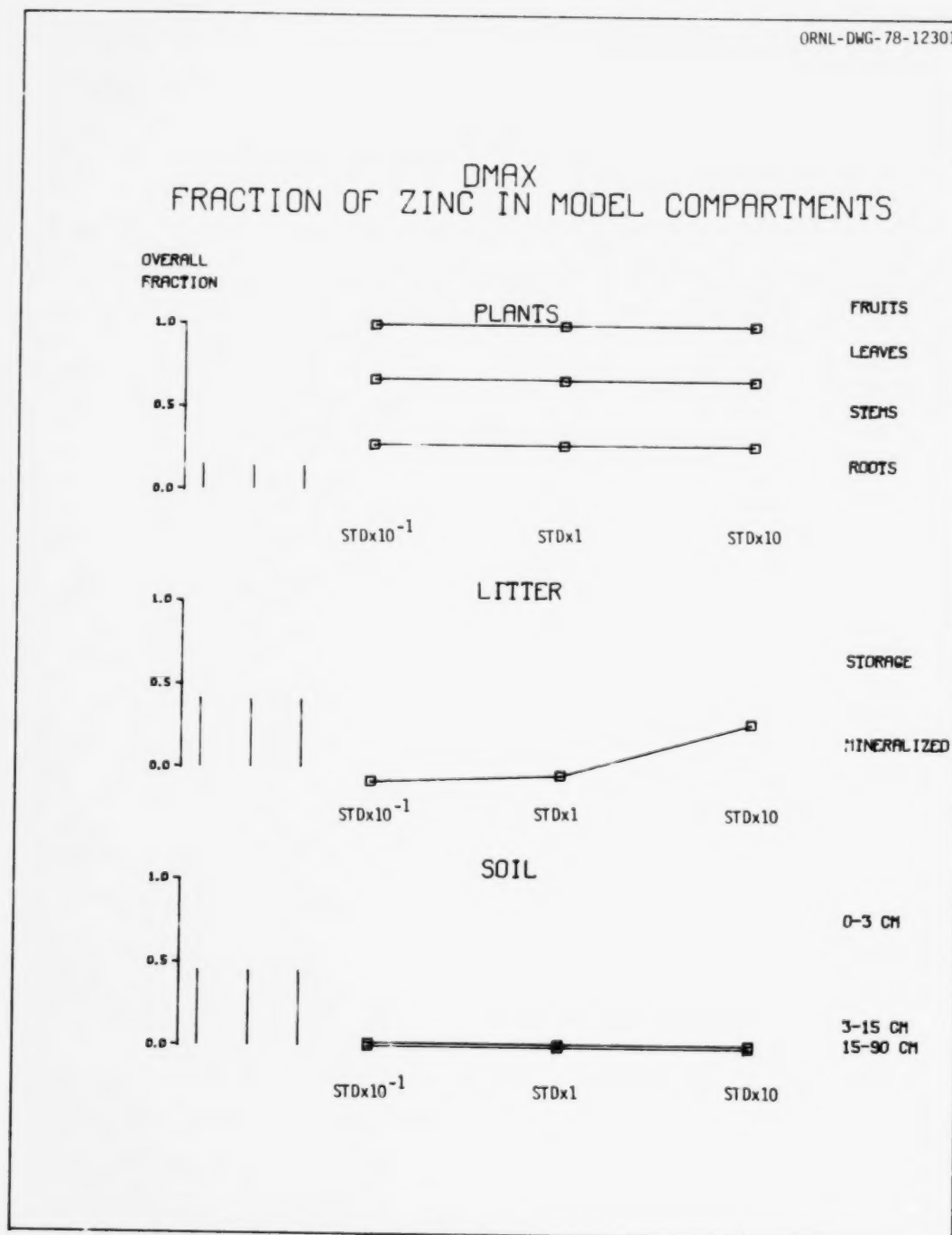


Figure 4.13. Relative zinc distribution in plant, litter, and soil components with change in litter decomposition rate constant (D_{MAX}).

chemical content in litter. The litter mineralization of chemical was the most sensitive output response.

Internal Plant Parameters

Phloem resistances (LSPHLO, SRPHLO, SFPHLO). The rate of transport of sugar substrate between plant compartments is proportional to the substrate gradient and a phloem resistance factor (Dixon et al. 1976). Leaf-to-stem (LSPHLO), stem-to-root (SRPHLO), and stem-to-fruit (SFPHLO) resistances are all used in the CERES model. Increase in the leaf-to-stem phloem resistance reduced total growth and changed the relative biomass distribution of the plant. At higher resistances, there was greater leaf growth and lower stem growth. The differences in biomass resulted in corresponding changes in contaminant uptake (Fig. 4.14). Lower resistance caused greater phloem chemical movement which was indicated by a higher sulfur content in roots supplied from leaf uptake and also in the hourly zinc translocation rate.

Increase in the stem-to-root phloem resistance slightly reduced total growth and changed the relative biomass distribution. Total contaminant uptake was highest at the lowest resistance, and this was achieved through greater relative leaf uptake. Rather high leaf uptake (due to high standard value of PERM) and reduced transport to other plant components at high phloem resistance, caused leaf concentration to be high. Mortality of these leaves increased the litter contaminant level and the rate of zinc mineralization. Thus a stem-root property influenced chemical dynamics in litter. The phloem translocation of chemicals was directly influenced by change in phloem resistance and this was shown in the output responses. Sulfur dioxide gas uptake was

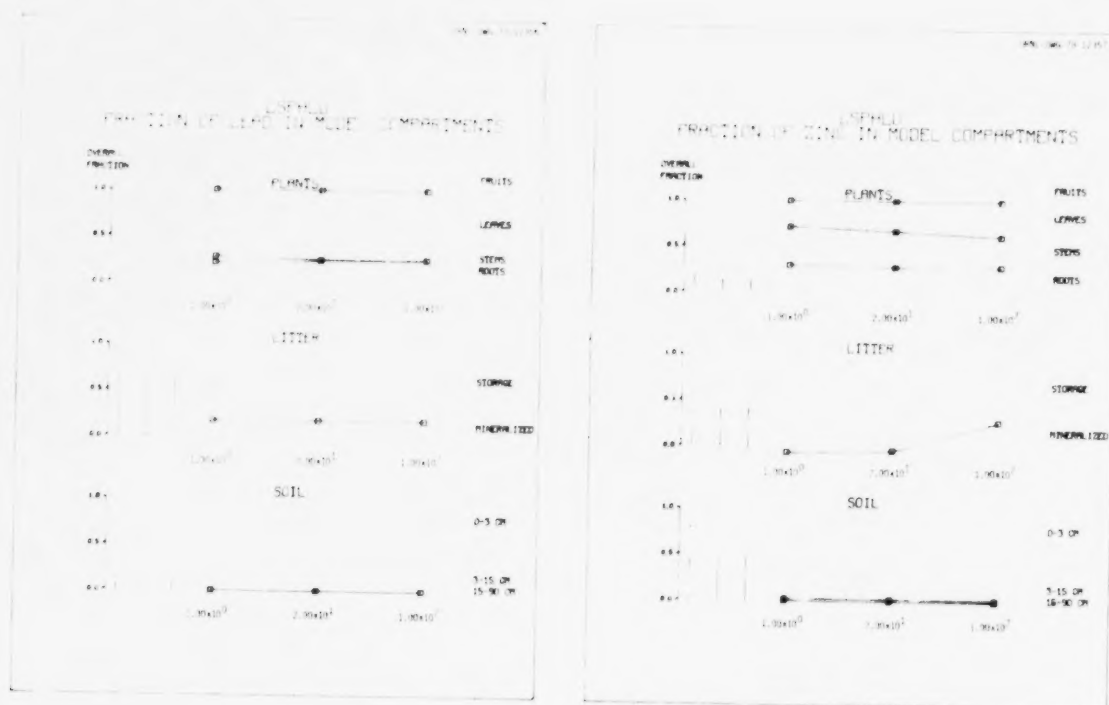


Figure 4.14. Relative chemical distribution in plant, litter, and soil components with change in leaf-to-stem phloem resistance (LSPHLO) for (a) lead, and (b) zinc.

influenced by LSPHLO. The zinc content in the root litter was very sensitive to both LSPHLO and SRPHLO.

Standard respiration rates (RRESTD, SRESTD, LRESTD, FRESTD).

Respiration rates determine the loss of sugar substrates from the plant parts. The total respiration of each plant compartment varied directly as a Q_{10} function of temperature and the compartment biomass. Input values for tissue respiration rates at given temperatures were used for the Q_{10} functions. Total biomass decreased with increase in the standard root respiration rate and the relative proportion of roots decreased.

The root contaminant uptake increased as the root biomass increased for decreased standard root respiration rate (Fig. 4.15). At the two lower standard respiration rate values, zinc uptake became limited by the plant capacity for more zinc, and the plant compartments approached maximum zinc content. Lead uptake did not approach maximum capacity and the roots showed a preferential retention of lead for the highest standard respiration rate case. The large proportion of lead in leaves was due to leaf uptake.

Zinc uptake definitely decreased with greater stem respiration rates whereas there was little change for lead. Because of the decrease in stem biomass, a greater fraction of the zinc stayed in the roots and leaves. The mortality of leaves with high contaminant levels increased the litter contaminant mineralization.

Both leaf and fruit respiration rates had little effect on the overall fraction of contaminant in the plant. There was a small decrease in amount of zinc in the plant for higher leaf respiration

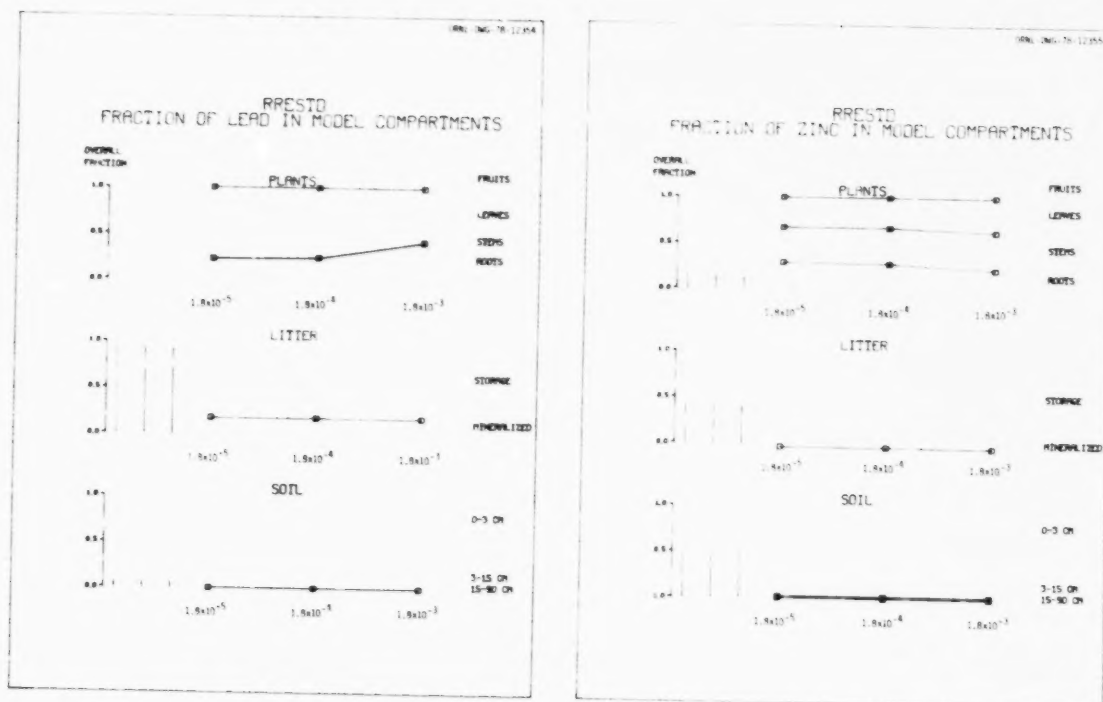


Figure 4.15. Relative chemical distribution in plant, litter, and soil components with change in standard root respiration rate (RRESTD) for (a) lead, and (b) zinc.

rate. The difference in respiration rates influenced a large number of plant and chemical output responses. The most significant responses to the RRESTD, SRESTD, LRESTD, and FRESTD factors are root leaf concentration, zinc content in leaf xylem, SO_2 uptake, and fruit zinc concentrations respectively. Changes in stem-to-root translocation of zinc was induced by changes in biomass for the different root respiration rates. Stem-to-root sugar translocation was highest for the largest standard stem respiration rate (Fig. 4.16).

Leaf area-to-weight ratio (ARI). The leaf area index is proportional to the area-to-weight ratio and is given by the product of leaf biomass and ARI. Therefore, the rates of net photosynthesis and transpiration are directly affected by varying ARI. Total plant growth increased with increase in ARI and there were changes in the proportion of plant parts. This, in turn, affected the concentration of the contaminants in the plant tissues. Relative lead and zinc concentrations in the plant were affected differently (Fig. 4.17). A ratio value of 50 is representative of thickened sun leaves, 100 is representative of mesic sun leaves, and 200 is representative of mesic shade leaves. Sulfur dioxide uptake, leaf sulfur concentration, zinc uptake by leaves, and leaf lead concentration were some of the most responsive outputs from the models.

Water potential effects (EI). A growth coefficient is used to simulate water stress effects on tissue (leaf, stem, root) growth. The coefficient is the value of an exponential function with a range from 0 to 1 which represents no growth or unaffected growth respectively. Input water potential values determining the sensitivity function

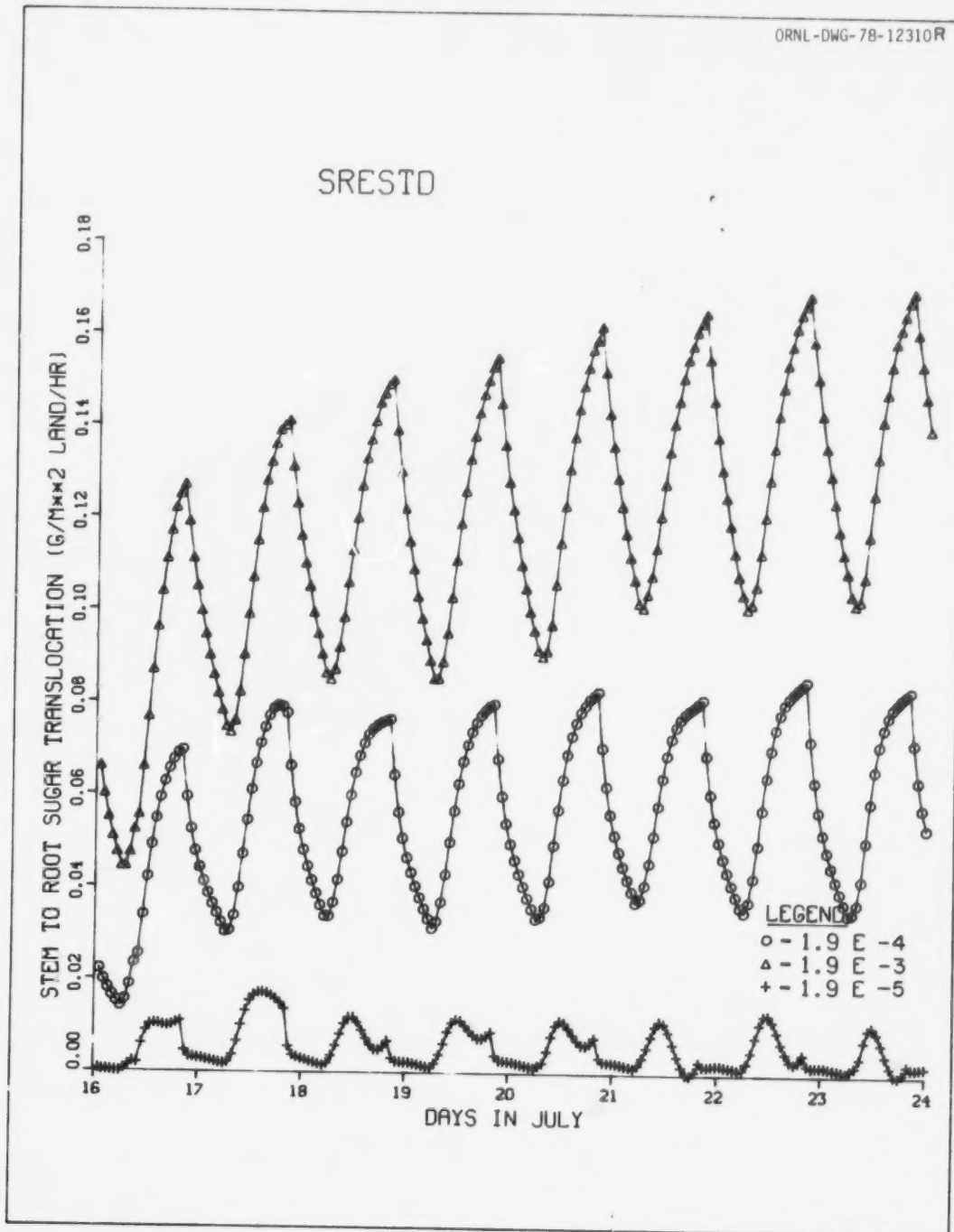


Figure 4.16. Influence of standard stem respiration rate (SRESTD) on sugar translocation in stem-to-root phloem.

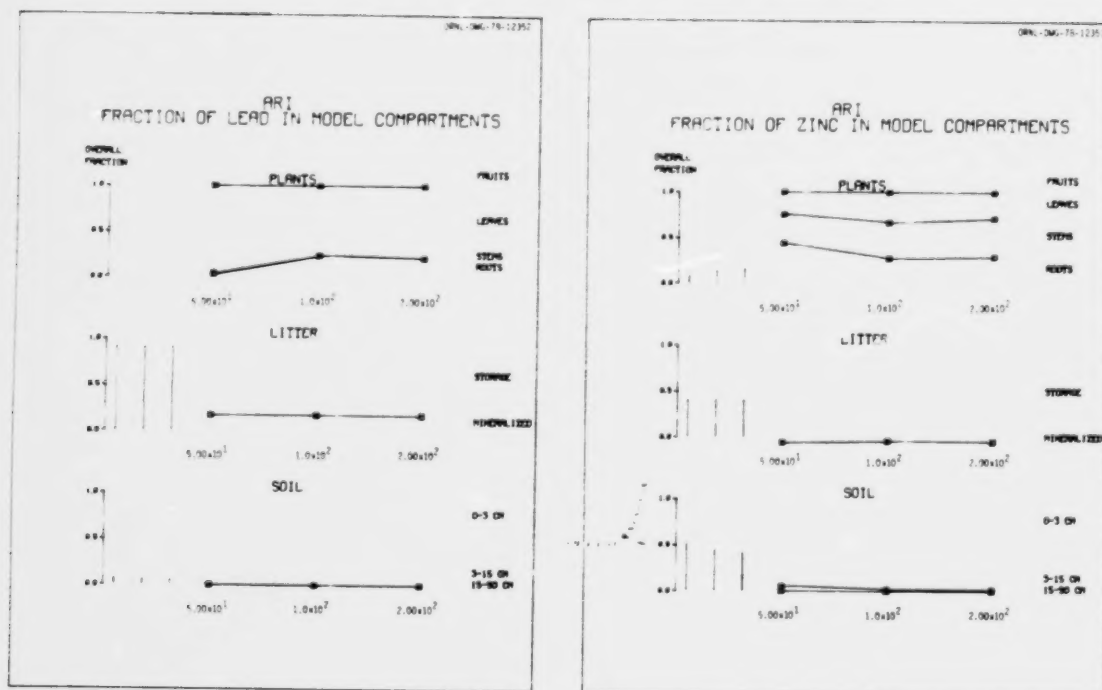


Figure 4.17. Relative chemical distribution in plant, litter, and soil components with change in leaf area weight ratio (ARI) for (a) lead, and (b) zinc.

include an initial potential at which tissue growth is reduced, the potential where tissue growth is reduced by one-half, and the potential where no further growth occurs (Dixon et al. 1976). Incremental values are added to each of these potentials in this test of water stress effects on overall plant growth, such that the greater the input potential the less growth is affected by the water potential stress. Thus total productivity was higher with reduced sensitivity to water effects. Stem growth was proportionally increased for decreased sensitivity, whereas the fraction of roots was not changed. Water potential influenced leaf growth more readily than other tissues and SO_2 uptake was the most sensitive output response. Stem biomass and chemical content in xylem tissues were also greatly influenced.

Root radius (R). The average root radius is used to determine root density and is a factor in the mass flow and diffusive uptake of soil contaminants. The direct dependence of root radius on uptake is difficult to determine because many of the factors in the uptake equations are dependent on R. For zinc, uptake during the sunlight period is increased with greater root radius. The root uptake for lead during the sunlight period, however, varies in exactly the opposite way (Fig. 4.18); there is more root uptake for the decreased root radius. These responses depend on the interaction of plant demand and the supply from the soil. The proportional contaminant distribution in the plant, litter, and soil components shows relatively little change with change in root radius. The most sensitive output responses to change in root radius were the chemical content in litter and the mineralization rates of chemical in the litter.

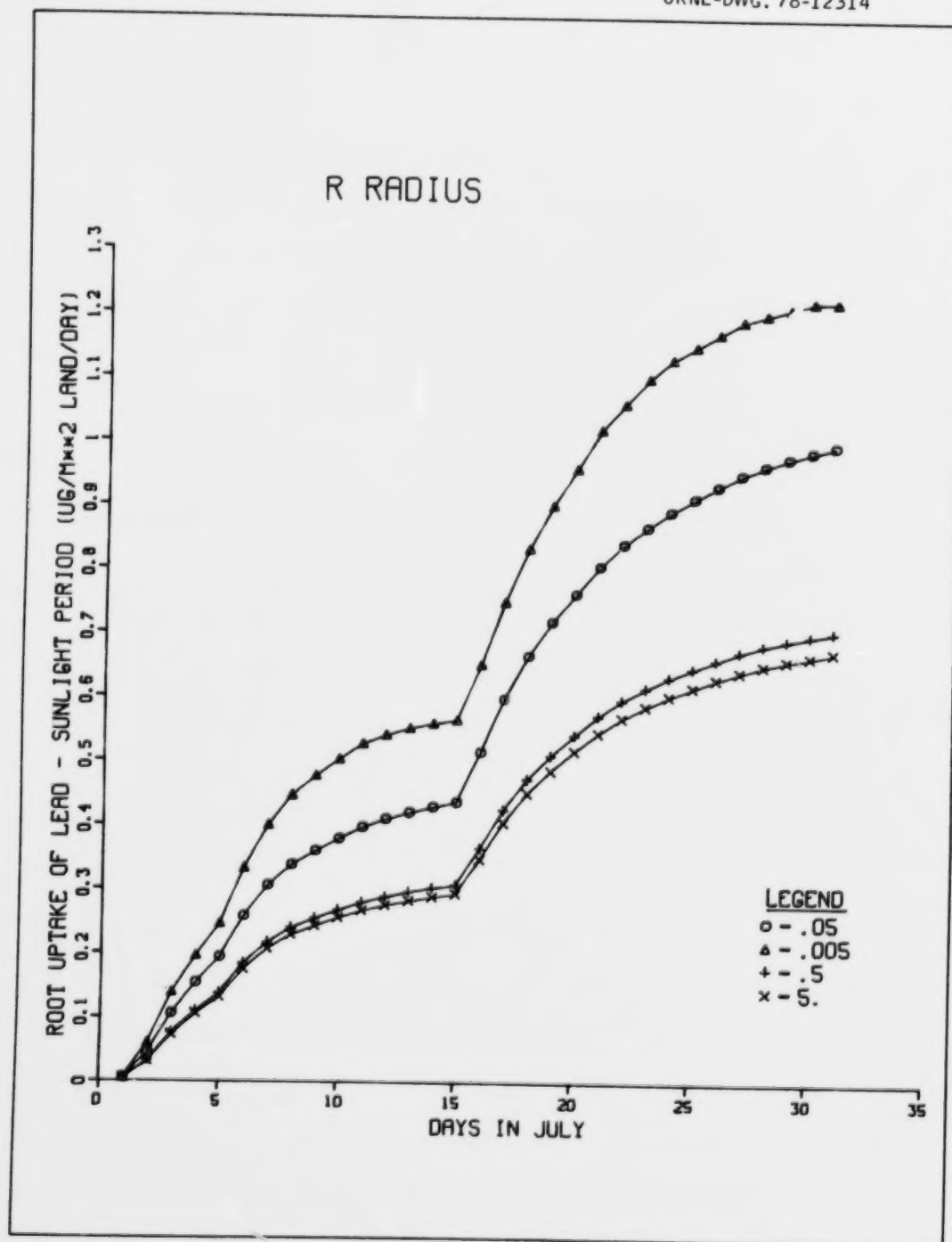


Figure 4.18. Influence of root radius (R) on root uptake of lead during the sunlight period.

Maximum leaf storage (L MAX). Each plant compartment has an upper limit to its storage value and the tissue growth rate is proportional to the difference between the current storage and maximum storage. The leaf maximum storage was reduced to test the sensitivity on overall plant response.

A reduction in total biomass as leaf maximum decreases is associated with a greater proportion of the biomass being located in the roots and stems. Lead uptake into the stem and root compartment changes reciprocally with change in L MAX (Fig. 4.19). The main lead uptake is primarily through the leaves, whereas root uptake accounts for the smaller lead content in the roots. At the lower biomass levels with lower L MAX values, the leaf lead content approaches a maximum. SO_2 uptake was the most significant model response to change in maximum leaf storage and surprisingly the zinc content of root litter was also very responsive.

Plant Boundary and Atmosphere Parameters

Leaf cuticle permeability (PERM). The rate of solute uptake across the leaf surface is dependent upon the solute concentration gradient, leaf cuticle thickness, and the leaf cuticle permeability. The permeability factor is one of the most sensitive parameters tested, especially in relation to contaminant movement.

The change in leaf uptake causes a change in the amount deposited on the litter surface. The increase in the overall fraction of contaminant in plants is accompanied by an overall decrease of the amount in the litter (Fig. 4.20). Zinc has a higher solubility than lead and this is shown in the reduced litter zinc content and greater soil content.

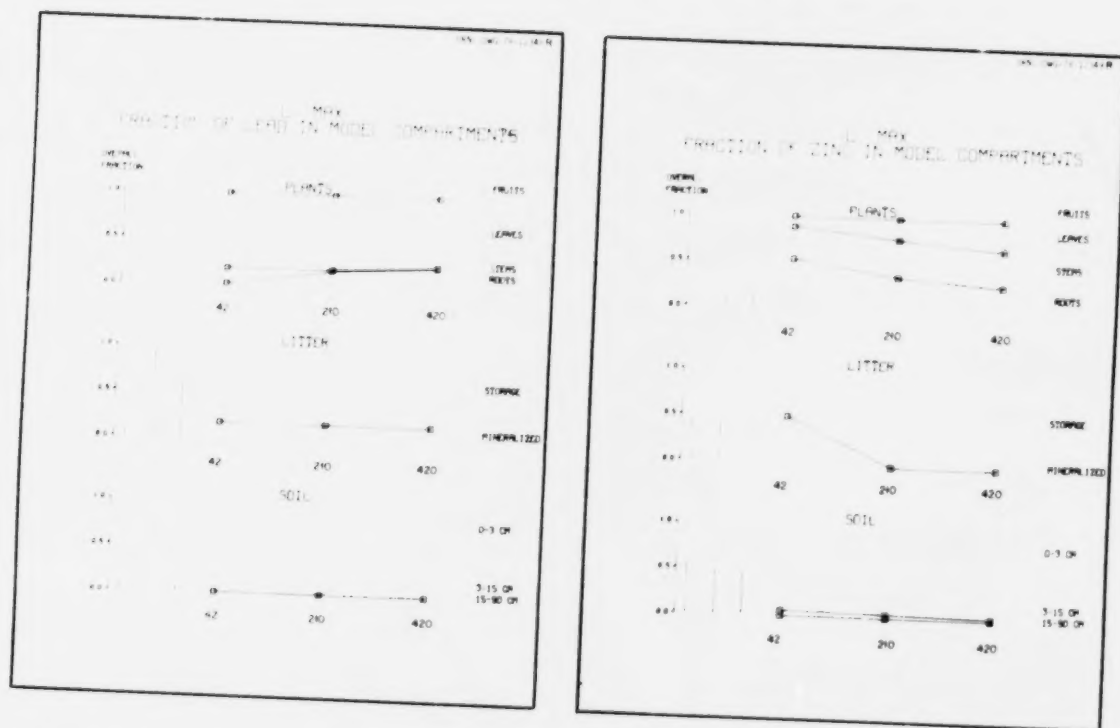


Figure 4.19. Relative chemical distribution in plant, litter, and soil components with change in maximum leaf storage (L MAX) for (a) lead, and (b) zinc.

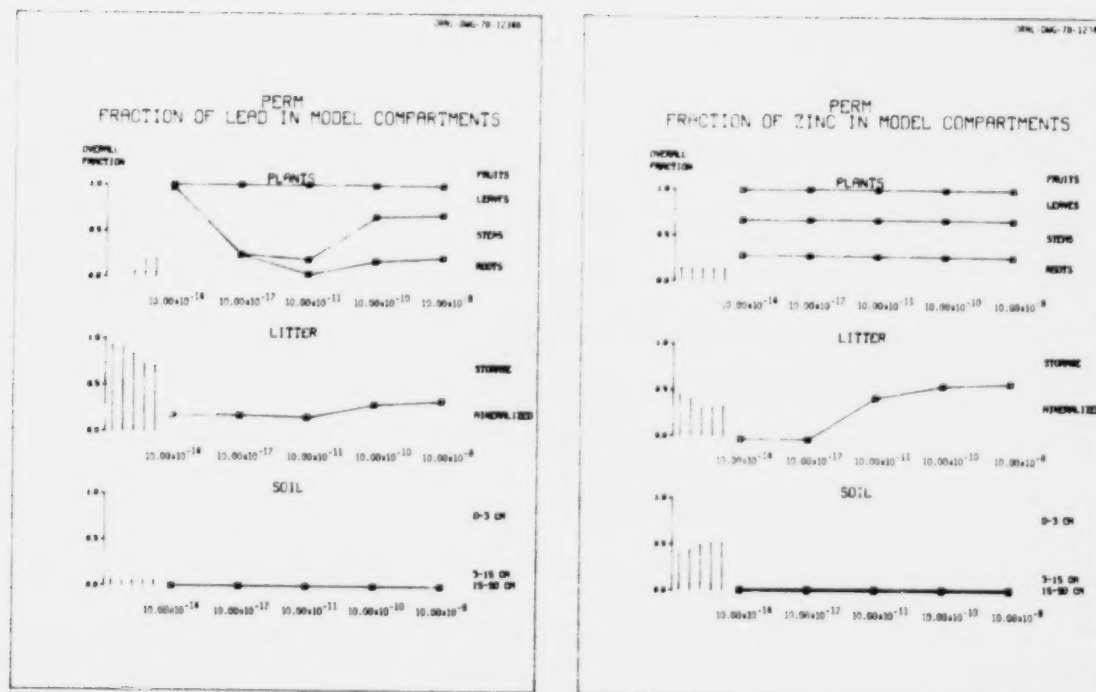


Figure 4.20. Relative chemical distribution in plant, litter, and soil components with change in leaf permeability to chemicals (PERM) for (a) lead, and (b) zinc.

Leaf uptake of zinc with increasing permeability contrasts with a corresponding decrease in root uptake of zinc. Both the lead and zinc leached from leaves were greatly influenced by change in PERM. Chemical transport within the vegetation and chemical accumulation in litter were responsive to the changes in leaf permeability.

Cuticle thickness (FILM). The solute uptake from the leaf surface is inversely proportional to leaf cuticle thickness. Therefore, the relationships depicting the fraction of contaminant in each of the compartments (Fig. 4.21) is almost completely opposite of Fig. 4.20. At high cuticle thicknesses, leaf uptake is diminished and the majority of the lead remains in the root system. Significant increases in zinc litter mineralization occurs at the low cuticle thickness values. The lead content of stem xylem, lead leached from leaves, and lead xylem transport from stem to leaf were highly influenced by change in cuticle thickness. The responses of zinc transport in the plant were suppressed because the plant had almost saturated its zinc uptake capacity.

Root solute conductivity (CONDOC). The actual plant solute uptake depends on the plant tissue capacity for additional solutes and the root solute conductivity. With increase in root solute conductivity, the overall fraction of contaminant in the plant increased (Fig. 4.22). The amount of zinc uptake through leaves increased with reduced root conductivity, causing greater accumulation in the leaf compartment. Alternatively when zinc uptake by roots was large, leaf uptake was reduced, allowing more contaminant to reach the litter surface and causing an increase in the amount mineralized. The model is very sensitive to the conductivity parameter. Zinc and lead content in the litter

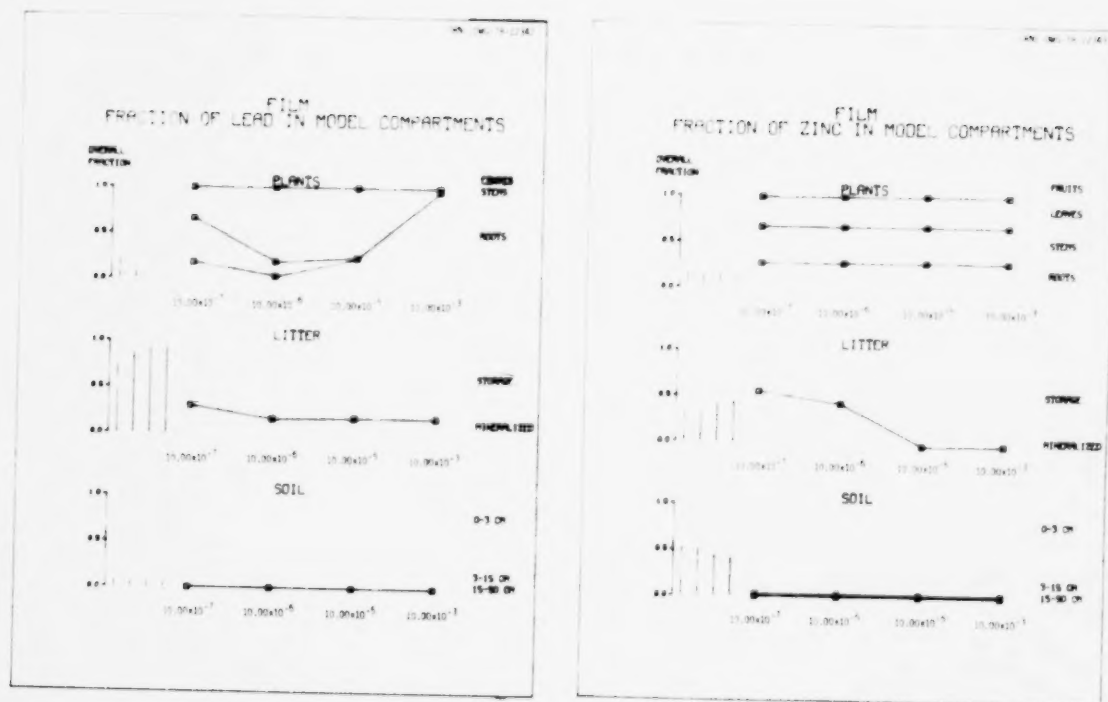


Figure 4.21. Relative chemical distribution in plant, litter, and soil components with change in leaf cuticle thickness (FILM) for (a) lead, and (b) zinc.

FRACTION OF ZINC IN MODEL COMPARTMENTS

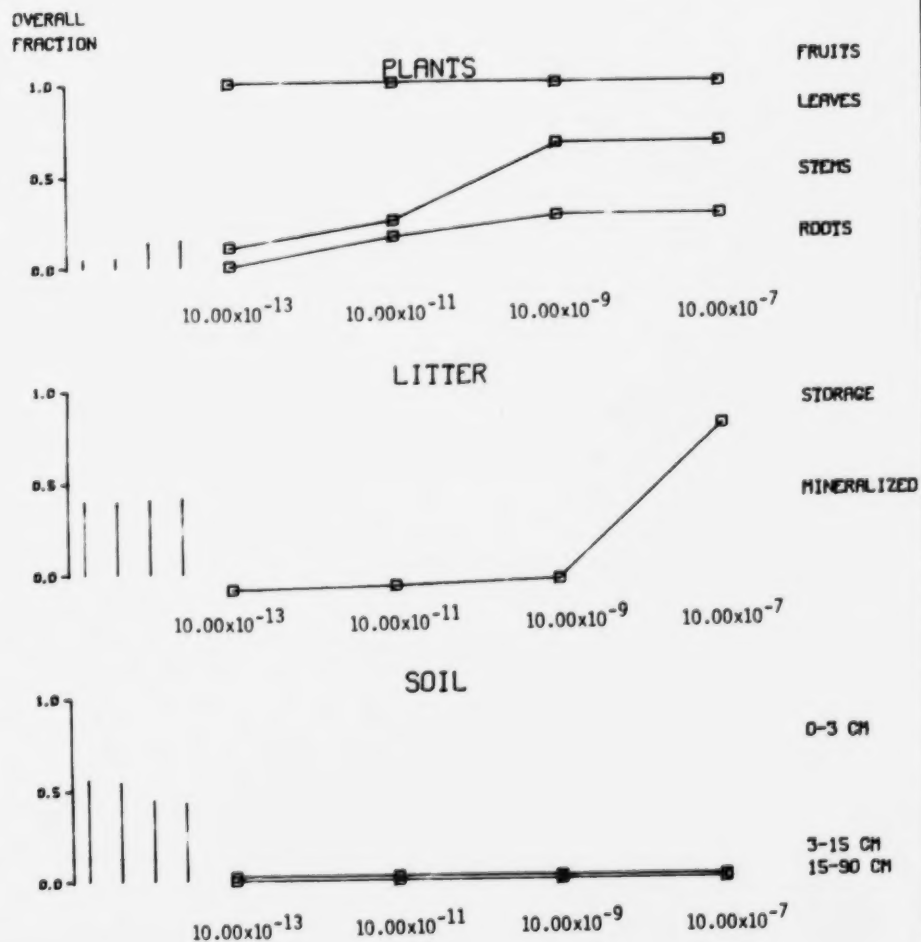


Figure 4.22. Relative zinc distribution in plant, litter, and soil components with change in root chemical conductivity (CONDUCT).

and the heavy metal mineralization rate were the most responsive outputs to change in root conductivity. The root lead concentration was the most responsive plant property.

External gas concentration (GASEX). The diffusive uptake of gases (SO_2 in this case) is calculated by determining the gas concentration differences between the leaf and the atmosphere. There was greater SO_2 uptake and greater sulfur translocation from leaves to stems with an increase in SO_2 concentration. The leaf compartment was the dominant sulfur sink for the conditions examined. The SO_2 uptake and sulfur dynamics in the vegetation were very responsive to change in external SO_2 concentration.

CO_2 concentration in ambient air (CO_2X). In the plant growth model, net photosynthesis depends on the CO_2 gradient from the chloroplast to the ambient air (Dixon et al. 1976). Increasing the ambient CO_2 concentration affects photosynthesis and causes an increase in plant growth. The greatest proportion of the increase is divided between the roots and stems. As a result, a slight increase in the overall fraction of zinc in the plants was obtained (Fig. 4.23). Chemical uptake and transport was influenced by change in atmospheric CO_2 concentration. The uptakes of SO_2 and zinc were the most responsive model outputs. Stem biomass was also significantly influenced.

4.4.1.3 Discussion

A subjective summary of parameter effects on the model output (Table 4.37) identifies five of the fifteen parameters as highly sensitive factors in the model behavior. These are the chemical distribution

CO₂X FRACTION OF ZINC IN MODEL COMPARTMENTS

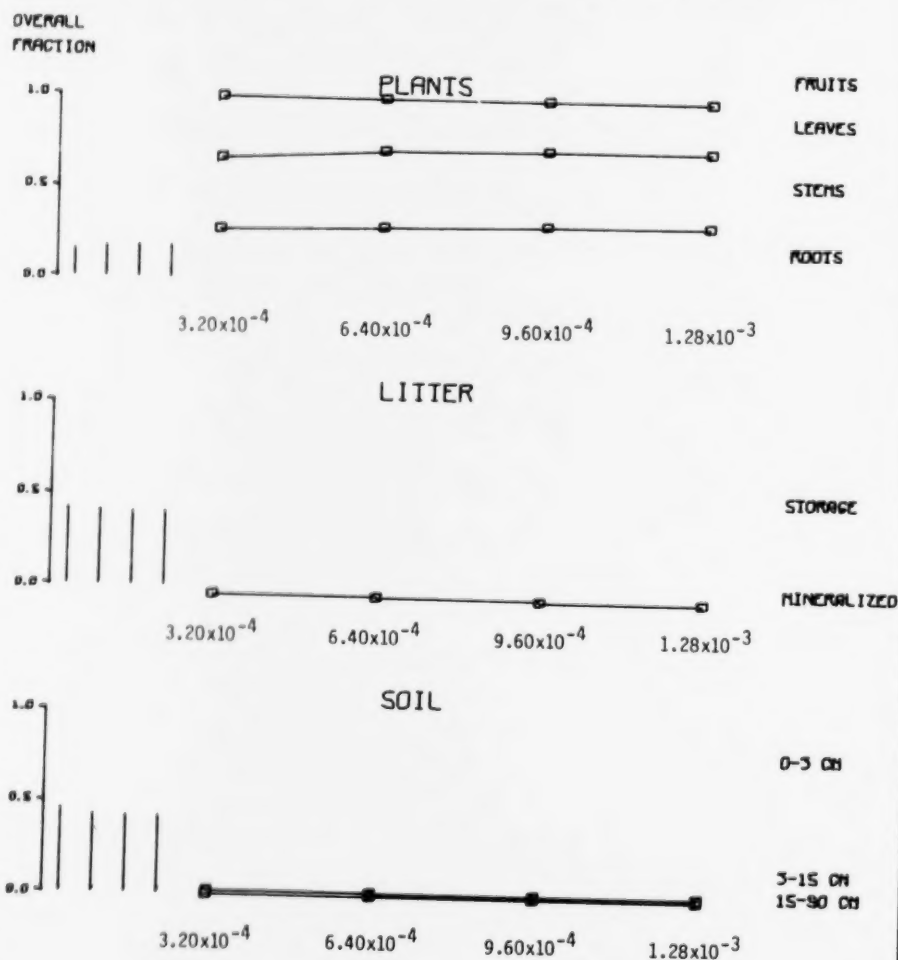


Figure 4.23. Relative zinc distribution in plant, litter, and soil components with change in atmospheric CO₂ (CO₂X).

Table 4.37. Subjective evaluation of parameter effects on total chemical level and distribution in soil, litter, and plant components^a

Increase in parameter	Total chemical level						Chemical distribution					
	Soil		Litter		Plant		Soil		Litter		Plant	
	Pb	Zn	Pb	Zn	Pb	Zn	Pb	Zn	Pb	Zn	Pb	Zn
K _d	+	++	0	-	-	-	-	--	0	+++	+++	++
SP	+++	++	---	--	+	0	0	0	+++	+++	+	0
DL	+	+	0	0	-	-	0	0	0	++	++	++
DMAX	0	0	0	0	0	0	0	0	±	±	0	0
PHLO	0	0	0	0	0	-	0	0	0	++	±	±
RESTD	0	0	0	0	0	0	0	0	0	0	++	±
ARI	0	-	0	0	0	+	0	±	0	0	±	±
EI	0	-	0	0	0	+	0	±	0	0	±	±
R	0	0	0	0	0	0	0	0	0	±	±	0
LMAX	0	-	0	-	0	++	0	±	0	++	±	++
PERM	0	++	--	--	+++	0	0	0	±	+++	+++	0
FILM	0	--	++	++	---	0	0	0	±	+++	+++	0
CONDC	-	--	0	0	+	++	0	0	0	+++	+++	++
GASEX	0	0	0	0	0	0	0	0	0	0	0	0
CO2X	0	-	0	0	0	+	0	0	0	0	±	±

^aQualitative ranking:

- +++ large increase
- ++ fair increase
- + increase
- 0 no change
- small decrease
- fair decrease
- large decrease

coefficient in soil (K_d), the chemical solubility (SP), the leaf permeability to chemical (PERM), leaf cuticle thickness (FILM), and the root conductivity to chemical (CONDOC). The K_d , SP, and FILM terms can be fairly readily measured; however, this is not the case for the plant characteristics that determine the rate of chemical uptake at its leaf and root boundaries (PERM, CONDOC). Estimates of these characteristics can be obtained by tuning the models to results from experimental uptake studies (Luxmoore and Begovich 1979).

Two parameters (DL, R) showed surprising results by generating opposite responses for zinc (soluble, mobile) and lead (less soluble and less mobile) movement within the plant.

A consistent pattern obtained in the model output was an increase in litter mineralization in response to factors that increased the chemical content in the plant leaf tissue. These include the phloem resistance (LSPHLO, SRPHLO), standard tissue respiration rate (SRESTD), maximum leaf weight (LMAX), leaf permeability (PERM), leaf cuticle thickness (FILM), and root conductivity (CONDOC). Chemical dynamics in the litter system also showed responses to the chemical distribution coefficient (K_d), chemical solubility (SP), chemical diffusion coefficient (DL), litter decomposition characteristic (DMAX), and root radius (R), as shown by the response and sensitivity coefficients. Some of the indirect relationships would not have been expected but became apparent through modeling methods. The study also suggests that monitoring of litter systems (chemical content, chemical mineralization) can provide one detection method of plant response to chemical perturbation.

Sulfur dioxide uptake from a chronic atmospheric concentration (2×10^{-8} ml/ml) was found to be very responsive to change in several plant properties. Reduced leaf-to-stem phloem resistance (LSPHLO), reduced stem-to-root phloem resistance (SRPHLO), reduced leaf respiration (LRESTD), increased leaf area weight ratio (ARI), reduced water stress sensitivity (EI), increased leaf storage (L MAX), and increased atmospheric CO_2 (CO_2X) all resulted in an increased SO_2 uptake by the vegetation. These effects were all the result of an increased sink for sulfur by increased growth associated with the change in parameter value.

Much information is contained in the recent report by Begovich and Luxmoore (1979) and has not been commented on; nevertheless, the data can be used as a reference to examine further any model responses of interest.

4.4.2 Simulation of Chemical Transport in a Deciduous Forest Using Microcosm Calibration

This work compares the results from a six-year simulation of heavy metal transport in a deciduous forest with field data collected during the sixth year after commencement of lead mining and smelting at the new lead belt in southeastern Missouri. The study does not attempt to "validate" a model per se, but seeks to identify a role for simulation in testing protocols and to evaluate some of the advantages and disadvantages of a combined microcosm and modeling approach through a test case application.

4.4.2.1 Tree Microcosm Study and Model Parameterization

Six intact soil blocks (45 x 45 x 25 cm depth), each containing one red maple (Acer rubrum L.) sapling, ground flora, and litter, were taken from a mesic forest location and reestablished in a greenhouse (Jackson et al. 1978). Following a two-month adjustment period, heavy metals (Pb, Cd, Zn, Cu) were added to the soil surface in an initial dose and in weekly treatments for 12 weeks. After 20 months the heavy metal content of the tree parts, soil, and litter were analyzed in a final harvest.

A 24-month simulation (October 1974-September 1976) was conducted with the coupled models for carbon, water, and heavy metal dynamics (Table 4.34). The soil system was characterized with representative hydraulic properties (Peters et al. 1970) and soil chemical properties (Sweeton, unpublished data). Initial conditions for the litter and plant dry weight (Jackson, personal communication) and heavy metal levels in plants, litter, and soil were obtained (Jackson et al. 1978). Meteorological conditions within the greenhouse were derived by modification of Oak Ridge records for the study period, according to inside-to-outside weighting factors determined experimentally. Daily maximum air temperature was increased by 11.7°C and the minimum was increased by 2.2°C. Daily average dew point temperature was increased by 2.2°C and solar radiation was reduced by 50%. The watering regime of the microcosms was represented by equivalent precipitation amounts in the model, adjusted to eliminate canopy interception.

Initial model calibration was conducted on parameters and meteorological data that influenced total water loss. Watering records were

used to calculate annual evapotranspiration (ET) as the difference between the water applied and the total drainage from the microcosms. The estimated experimental ET was 148 cm/year. The initial simulations with parameter adjustments gave ET in the range 59 to 75 cm/year. Some of the adjustments were extreme and outside the range of published physiological data. Modification of the temperature and irradiation data for the greenhouse was also examined. The lack of agreement between the microcosm and the model in terms of water budget was probably related to poorly characterized microclimate in the greenhouse and physiological changes in an outdoor plant adjusting to a greenhouse environment. Advective heat may have been a major source of energy for ET that was not possible to characterize because the microcosms were located near an exhaust fan in the greenhouse. There was no way of meaningfully tuning the models to the microcosm water budget. The simulation gave results (Table 4.38) representative of outside water budgets for the Oak Ridge area (Luxmoore et al. 1978b).

Adjustment of the carbon budget of the plant and litter system was undertaken next, and reasonable agreement was obtained with the final harvest data (Table 4.38). The main parameters adjusted were the maximum stem and root storage which determined the overall biomass of the tree. The litter decomposition rate was also adjusted to give a total mass that was comparable with the measured value (Table 4.38).

The final phase of adjustment was to obtain agreement between the observed and simulated chemical data. The chemical solubility was adjusted such that the amount of observed and simulated chemical in the litter layer was in close agreement (Table 4.38). The chemical

Table 4.38. Comparison of water, carbon, and chemical data for the microcosm and modeling studies

	Microcosm	Model						
Water								
Drainage (cm/year)	6.5	93.0						
Evapotranspiration (cm/year)	148	60.9						
Carbon (final harvest)								
Leaf dry wt (g/m ²)	281	284						
Branch + bole dry wt (g/m ²)	1188	1159						
Litter dry wt (g/m ²)	1159	1151						
	Pb Cd Zn Cu	Pb Cd Zn Cu						
Chemical (final harvest) ^a								
Total plant uptake (μg/m ²)	153,000	2,400	85,000	3,050	162,000	2,300	77,000	3,100
Root concentration (μg/g)	12	0.2	57	0	25	0.4	13	0.5
Stem concentration (μg/g)	49	0.5	47	0	46	0.7	23	1.0
Leaf concentration (μg/g)	321	6	28	12	198	2.7	95	3.5
Litter concentration (μg/g)	9,000	80	485	335	15,681	107	127	515
Soil concentration (μg/g)								
0-5 cm	1,075	9	30	12	1172	15	87	10
5-10 cm	292	3	36	7	86	2.1	20	0.1
10-25 cm	451	3	13	7	1.6	0.1	1.0	0.0
Total soil chemical (μg/cm ²)	15,490	119	607	230	9,270	115	720	56

^aPlant, litter, and soil concentration represents the increase above the concentration in controls.

distribution coefficient (K_d) influenced the soil solution concentration and thus the rate of chemical transport between soil layers. This parameter was adjusted to modify the pattern of heavy metal transport through the soil system. The total plant uptake of heavy metals was adjusted by the appropriate change in the root solute conductivity. Finally the distribution of the chemical between plant parts was obtained through the introduction of parameters for chemical chelation within the plant. These parameters maintained differential levels of heavy metals in the soluble (thus transportable) form for each plant tissue (root, stem, leaf, fruit). The final agreements and disagreements in the chemical levels (Table 4.38) were accepted as adequate for the purposes of this study.

The tuning process proceeded by the adjustment of parameters sequentially from the independent to the more and more dependent variables, providing a stepwise process of adjustment. This process is such that it is difficult to complete unless some predefined optimization routine can be used. Parameter adjustment did not result in minimum statistical deviation between observed and simulated results.

The tuned parameter values may be viewed as apparent values in which the complexity of phenomena in the microcosm system is synthesized into a set of apparent values as far as the relatively simple model structure is concerned. Because the water and carbon fluxes in the microcosms are not related to those at the Crooked Creek Forest, none of the water and carbon parameter values for the microcosm were transferred to the Crooked Creek application. Four chemical parameters tuned to the microcosm were used in the final choice of chemical parameters

for the Crooked Creek test application. These parameters included the distribution coefficient (K_d), chemical solubility, root solute conductivity, and plant chelation factors. Both the distribution coefficient and chemical solubility can be readily measured by laboratory procedures; however, the tuned values can differ from this measurement, due to inadequacy of models to represent all significant phenomena. The tuning process derived values that represent the apparent value for microcosm phenomena according to the model representation. Root solute conductivity is a highly sensitive parameter and not readily determined experimentally. The microcosm data are ideal for estimation of this parameter. Initial tuning runs showed that there were significant differences in the uptake of each heavy metal, and the model was changed to allow a specific conductivity value for each chemical. The original model structure resulted in preferential chemical accumulation in vegetation in the tissue chemical uptake (e.g., roots). The microcosm study showed that chemicals moved readily in plants, with highest concentration occurring in leaves. A chelation parameter was introduced into the model and tuned to reflect the experimental data.

4.4.2.2 Field Study and Test Case Simulation

Heavy metals (P, Cd, Zn, Cu) released from a lead mine and smelter complex into a deciduous forest in southeastern Missouri have resulted in a reduction in litter decomposition rate (Watson et al. 1976) and a change in the nutrient cycling fluxes (Jackson and Watson 1977). These field studies conducted on the Crooked Creek Watershed during 1974 and early 1975 report effects of continued heavy metal deposition since the industrial facilities commenced operation on the watershed boundary in

late 1968. Simulation of heavy metal transport and accumulation in the soil-plant-litter system at 0.4 km from the industrial source was conducted for the test case comparison.

Meteorological and precipitation records for the seven-year period, October 1968 to September 1975, were coded into the model format from available records at surrounding meteorological stations. Hourly precipitation was mainly obtained for the Viburnum station which is 10 km north of the study area. Average wind speed, maximum and minimum daily temperature, and average dewpoint temperature were obtained from both onsite records and from Salem, Missouri (38 km west).

Soil hydraulic properties were determined onsite with the instantaneous profile method by Doll (1976), and these data were used as inputs to the soil-plant-water flow component model. Parameters representing second growth oak (Quercus alba L., Q. velutina Lam.) forest were the same as previously used in simulations by Dixon et al. (1976) in their application of the CERES forest growth model to Crooked Creek Watershed. The mean deposition rate of heavy metals from the industrial operation was 189, 1.26, 16.2, and 7.2 $\mu\text{g cm}^{-2} \text{ month}^{-1}$ for Pb, Cd, Zn, and Cu, respectively (Begovich and Jackson 1975). Data for the exchange capacity and bulk density for the soil profile were also taken from Begovich and Jackson (1975).

The simulation results for chemical transport obtained with the data set tuned to the microcosm results showed almost no agreement with the experimental data obtained from Crooked Creek Watershed (Table 4.39). The only exception was the cadmium and copper concentration results for the A1 soil layer. The 2.4-fold difference in evapotranspiration and

Table 4.39. A comparison of initial simulation, experimental data, and simulation results from this study for Crooked Creek Watershed

	Simulated ^a	Experimental ^b	This study
Total litter mass (g/m ²)	2448.0	3600.0	4972.5
Litter concentration (ppm)			
Lead	51000.0	72000.0	2.76
Cadmium	5.3	150.0	0.017
Zinc	121.0	2350.0	0.23
Copper	375.0	1400.0	570
Leaf tissue concentration (ppm)			
Lead	170.0	467.0	0.436
Cadmium	10.0	3.9	0.004
Zinc	50.0	47.0	0.151
Copper	10.0	10.0	0.061
Root tissue concentration (ppm)			
Lead	1150.0	2400.0	3.35
Cadmium	52.0	46.0	0.04
Zinc	52.0	48.0	1.28
Copper	10.0	6.0	0.50
Stem tissue concentration (ppm)			
Lead	27.0	680.0	3.39
Cadmium	9.0	3.9	0.04
Zinc	35.0	30.0	1.38
Copper	18.0	13.0	0.55
Soil concentration A1 layer (ppm)			
Lead	271.0	334.0	2561
Cadmium	4.4	3.55	4.03
Zinc	52.0	25.0	55.3
Copper	46.0	18.0	17.5

^aFrom Munro et al. 1976.

^bFrom Bondietti et al. 1976.

drainage in the microcosm tuning exercise cannot account for the differences in the field test case because values of root solute conductivity would have been lower at higher water fluxes into roots to give the same total chemical uptake. It is evident that the chemical parameters obtained for a seedling tree in a greenhouse environment cannot be directly applied to mature forest and that the applicability of models and microcosms in testing protocols requires careful evaluation.

The heavy metal concentration in litter was much lower for Pb, Cd, and Zn in this study, which was mainly the result of high chemical solubility values. However, Cu had a lower solubility and the litter concentration was somewhat closer to the experimental result than that obtained in the Munro study. The heavy metal concentration in plant tissues in this simulation study was very much lower than the earlier simulation which gave results that were in closer agreement with the experimental data. The root solute conductivity was three to five orders of magnitude smaller in the tuned data set than was that used in the Munro et al. (1976) study. The large difference between the experimental and simulated plant chemical concentrations shows that the root conductivity parameter must also be determined for the real world conditions.

The distribution coefficients used in this simulation were much lower than those used by Munro et al. 1976, resulting in greater chemical mobility through the soil. There was closer agreement between the simulated and experimental data for the soil comparisons than between those for the plant. The inference is made that the apparent K_d values derived from microcosm studies provide a better representation

of the effective chemical distribution between solid and liquid phases than a laboratory determination of the K_d for the soil of interest. This makes sense if one considers that other phenomena (e.g., chelation) influence chemical mobility, and these are not accounted for in the laboratory determination of K_d but are included in the derived value from microcosm studies.

4.4.2.3 Discussion

A recent workshop proceedings on terrestrial microcosms (Gillett and Witt 1979) recommended that mathematical models using "basic physiochemical and biologic data" be tested in microcosm studies with the results in turn being used to develop models for environmental behavior of chemicals. This has been done in the present study and modifications to the models are believed to increase the real world utility of the codes. The same workshop identified the need to relate quantitatively the data from microcosms to chemical behavior in natural ecosystems. This need was addressed in this study with the simulation models providing the means of quantitatively relating microcosm data to real world chemical behavior. Our test case application showed that we failed completely in this task, not because of inadequate model algorithms but by the choice of parameter values derived from the microcosm data. Clearly the simulation is limited by the field relevance of the microcosm data set. It was a gross extrapolation to expect that parameter values derived for a sapling tree grown under greenhouse conditions should be relevant to a Missouri deciduous forest. The tuned model data set could perhaps be more readily used to evaluate heavy metal behavior in a tree nursery-greenhouse facility.

Several advantages of the combined use of modeling and microcosm methodologies may be identified:

- (1) Microcosm data can be used to evaluate model algorithms and structure.
- (2) Models may also be validated by microcosm data sets in which the model is first tuned to one data set and then the model simulation compared with another data set.
- (3) Microcosm data provides one means of model parameter estimation; however, the parameter values may not be representative of natural ecosystems.
- (4) The combined methodologies reduce biological complexity (microcosm) to a simplified representation (model), allowing the generation of time extrapolation insights.

The limitations of combined microcosm and modeling methodologies in protocols for assessing real world phenomena relate to the relevance of the methods to the real world behavior. The modeling exercise could be at a double disadvantage by being limited by both algorithm simplification of the real world and errors in input parameter estimation.

The exercise undertaken in this study is a first step in the development of a combined microcosm-modeling protocol. The study showed that the model could be tuned to microcosm systems, and we believe that simulation of real world phenomena is possible with suitable sources of input parameter values. Field studies or "in situ" microcosms are more likely to provide relevant input data for models than indoor microcosm studies. Further, the manual stepwise tuning process used in this study should be replaced with some computer parameter optimization procedure

to reduce subjectivity. This has been done for a parametric hydrology model (Fields and Watson 1975) and the approach can be readily adapted to the models used in this study.

4.5 DISCUSSION

4.5.1 The Role of Terrestrial Microcosms in Contaminant Research* (B. S. Ausmus, D. R. Jackson, and P. Van Voris)

4.5.1.1 Introduction

Need for Accuracy of Screening Techniques

Microcosm experimental units are confined, replicable tools which have been developed in response to recent legislative requirements contained within the Toxic Substances Control Act (P.L. 94-469) and the Resource Conservation and Recovery Act (P.L. 94-580). These Acts require testing of potentially hazardous substances resulting from manufacturing, processing, and waste disposal. Criteria for testing apply to those substances (1) which may present an unreasonable risk to health or environment, (2) for which insufficient data exist for assessment of risk to be made, and (3) for which testing is required to develop data needed for assessment of risk. Substances deemed potentially hazardous to health or environment, or produced in substantial quantities (thereby increasing the risk of exposure), can be required to be tested.

The criteria for testing remain unclear (P.L. 94-469) and must be approved by the Environmental Protection Agency (EPA) before use.

*Taken from Ausmus, B. S., Jackson, D. R., and P. Van Voris. 1979. The Accuracy of Screening Techniques. pp. 123-130. IN James M. Watt and James W. Gillett (eds.), Industrial Microcosms and Environmental Chemistry. National Science Foundation (NSF), Washington, D. C.

Procedures for use as the initial screening, termed Level 1 Environmental Assessment: Biological Tests (Duke et al. 1977), have three goals. These are: (1) provision of preliminary environmental assessment data; (2) identification of problem areas; and (3) collation of data needed to prioritize further assessment activities. Two additional levels of assessment remain to be developed. Level 2 studies will allow more specific studies investigating rates of transport and levels of effects. Level 3 studies will be designed to discern the exact mechanisms disrupted by the substances, allowing appropriate management control or elimination of problems.

Under legislation, research to develop these screening tools can be sponsored by EPA. Specific testing of the substances, however, are the responsibility of industry. The initial screening reports accepted by EPA are expected to be the 'pacesetters' for following reports. The EPA will require only enough data to reach regulatory decisions and will update acceptable screening procedures as the science (or art) progresses. These updates will not jeopardize the acceptability of the earlier-prescribed screening procedures (P.L. 94-469).

Microcosm Screening Techniques

Microcosms have been used to address several levels of biological complexity, including cellular, tissue, organ, organismal, population, community, and ecosystem (Witherspoon et al. 1976). The gamut of experimental units, then, includes pure cultures to excised, intact portions of terrestrial landscapes (Table 4.40).

Microcosm studies are being used as experimental tools to assess chemical transport, fate, and effects upon ecosystem components,

Table 4.40. Uses that various microcosm units have for specific screening applications

Screening Application	Microcosm type				
	Bioassay	Biomagnification	Strata cores	Excised systems	In situ studies
Total transport				X	X
Total fate					
Distribution				X	X
Accumulation				X	X
Strata transport			X	X	X
Accumulation					
Within strata			X	X	X
Trophic transfer		X	X	X	X
Target species	X	X	X	X	X
Target communities		X	X	X	X
Process disruption			X	X	X
Process sensitivity			X	X	X
System disruption				X	X
System sensitivity				X	X
Mechanisms of disruption	X	X	X	X	

specifically populations, and trophic web members (Metcalf et al. 1973, Booth et al. 1973, Focht and Alexander 1970, Francis et al. 1975). Within these studies, a variety of artificial and homogenized substrates have been used to construct experimental units (Witherspoon et al. 1976). However, these studies address ecosystem components rather than ecosystem processes, such as nutrient cycling and carbon metabolism. The effects upon an ecosystem's ability to compensate for and to recover from chemical stress cannot be inferred from studies of ecosystem components since the interconnections among components to form an ecosystem are not necessarily additive (O'Neill et al. 1975, O'Neill et al. 1977).

The criteria for microcosm acceptance as screening tools include their demonstrated replicability, reproducibility, and accuracy or realism of the data produced. These will provide initial quality control for screening procedures, and quantitative background information for regulatory decisions.

Witherspoon et al. (1976) discuss the complexities, applications, measured parameters, reproducibility, and realism in their review of microcosm research. They summarized the suitability of various designs for assessment of chemical accumulation sites, mutualistic and antagonistic processes, transport of the substances, and identification of critical problems in microcosm use. Problems listed included: (1) lack of demonstrated applicability of transfer rates and distribution of substances within microcosms to ecosystems; (2) lack of comparability or reproducibility of microcosm experiments; and (3) lack of estimated confidence intervals around microcosm measurements of several parameters.

Objectives

We have examined several types of microcosm experimental units with the ultimate purpose of assessing their utility as analogs of terrestrial ecosystem processes. We had three research goals; (1) to construct terrestrial microcosms which can be quantitatively equilibrated, replicated, and reproduced; (2) to identify and nondestructively measure parameters in microcosms which are indicative of ecosystem response to and recovery from stress; and (3) to quantitatively relate microcosm results to ecosystems (Table 4.41).

This discussion focuses on three themes. These are: (1) parameters which can be nondestructively monitored in microcosms to assess the transport rates of a substance and concomitantly its effect upon ecosystem processes; (2) the criteria for and comparison of the replicability and reproducibility among microcosm types tested; and (3) the criteria for and comparison of the accuracy of results among the microcosm types tested.

4.5.1.2 Measured Parameters

Criteria

There are five criteria which should be satisfied by the parameters chosen to measure in microcosm screening of potentially hazardous substances. First, the parameters measure should be indicative of terrestrial ecosystem function. That is, the measurements should be holistic rather than specific to ecosystem components, such as suspected target populations. Second, parameters should be used which can be nondestructively monitored throughout the experiment. This will allow detection

Table 4.41. Microcosm test systems, substances tested, and citations

Microcosm test system	Contaminant	Citation
Soil cores, no plants	Cd Pu, Cu, Cd, Zn As HCB	Ausmus and O'Neill, 1978 O'Neill et al., 1977 Jackson and Levin, 1979 Ausmus et al., in press
Soil core with plants	Cd Pu, Cu, Cd, Zn As	Van Voris, 1978 Jackson et al., 1978 In experimentation
Watershed, field	Pu, Cu, Cd, Zn	Jackson and Watson, 1977 Watson et al., 1976

of changes in parameters through time without the confounding which results from repeated removal of components of the microcosm units. Third, parameters monitored should allow rapid data acquisition at several intervals during the experiment. Daily or weekly measurements of parameters in experimental and control microcosms will increase statistical rigor of the screening experiment. Fourth, parameters should be chosen which allow both transport of the tested substance and effects on ecosystem processes to be simultaneously determined. Concentrations of dissolved organic carbon (DOC) and of the substance under study, for example, could both be monitored in leachates periodically taken from microcosms. Finally, parameters which are needed but which cannot be monitored can be determined on replicate microcosms sacrificed at time zero, and on all microcosms at time final. These include distribution of tested substance among ecosystem components and estimation of microbial, invertebrate, and plant biomass.

Monitored Parameters: Indices of Ecosystem Response to Stress

A natural candidate for terrestrial monitoring would be the rate at which macronutrients and carbon are exported from microcosms. Shugart et al. (1976) suggest that dissolved nutrients within soil water may be indicative of total ecosystem cycling of calcium. Microcosm studies, which treat these experimental units as model ecosystems, have shown that carbon and nutrient efflux from these systems are affected by chemical stress (O'Neill et al. 1977). Soil, because it contains the largest number of interacting components within terrestrial ecosystems, becomes the subsystem focused upon to provide initial screening

procedures. Because of the large number of interacting components in soil, detrimental effects are exhibited as increases in gaseous and dissolved carbon loss, and in increased dissolved macronutrient loss. This loss can be detected irrespective of the specific organisms and enzymatic pathways which were disrupted by chemical stress. The exact mode of effects may not be intuitively perceived. Therefore, focus upon a holistic index of ecosystem response to such an effluent would be desirable. Three studies, using soil only, intact soil-tree sapling units, and a watershed illustrate this point (Table 4.42). In each case disruption of nutrient cycling processes was detected prior to, or under less stress than, changes in population or community parameters. A recent aquatic microcosm study (Strange 1976) showed similar increased nutrient mobility in response to herbicide treatment.

Monitoring nutrient loss rates may not be necessary to detect ecosystem response to chemical stress. An index of nutrient status of soil under stressed conditions shows depletion of nutrient pools (Jackson and Hall 1978, Jackson et al. 1978, Ausmus et al. 1978). Figure 4.1 illustrates depletion of extractable nutrients soil following heavy metal application.

Measurements at Harvest: Distribution and Fate

While the rate of nutrient efflux and of tested substance efflux can be monitored throughout microcosm experiments, the distribution of the tested substance through microcosm components must be determined at the experiment's conclusion. Coupling monitored data with those at harvest, researchers can determine the mass balance of the tested substance and of the macronutrients of interest. The distribution of lead

Table 4.42 Population and system level parameters measured in soil microcosms, forest microcosms, and forest under control and chemical stress conditions (after O'Neill et al., 1977)

Level control and treatment		Population parameter	Values	System parameter	Values
Arsenic (NaHAsO_4)	0	ATP (ppm)	1.58 ± 0.33	Leached Ca (μg)	560 ± 36
	100 ppm		1.52 ± 0.30		714 ± 50
Arsenic (Na_2HAsO_4)	0	Bacterial density ($10^6/\text{g}$)	2.75 ± 0.13	Leached P (μg)	2.8 ± 0.5
	100 ppm		3.18 ± 0.41		21.0 ± 6.0
Heavy metals	0	Annual branch growth (cm)	347 ± 43	Leached Ca (ppm)	6.6 ± 0.4
	11 mg Pb/cm ²		346 ± 68		10.0 ± 0.8
Distance from lead smelter	21 km	Litter invertebrate diversity (Shannon Index)	2.3 ± 0.15	0.2 litter mass (g/m^2)	1008 ± 110
	2.0 km		2.5 ± 0.23		1595 ± 171

in forest microcosm components at the end of a 20-month experiment is summarized in Table 4.3.

Measurements at Harvest: Biological Composition

Detection of hazardous substance effect upon biota can also be made at experiment conclusion. Biomass differences between treated and control microcosms may indicate population shifts or community degradation due to chemical stress. Table 4.43 shows three indices of soil microbial populations measured at the conclusion of an experiment. Control microcosms had significantly greater fungal populations than microcosms which had been contaminated with a complex effluent of Pb, Zn, Cd, and Cu (Ausmus et al. 1978).

4.5.1.3 Replicability, Reproducibility

Criteria

In order to establish quality control of screening procedures three criteria must be met. First, the parameters must be predictable through the experimental period. Usually, this requirement necessitates equilibration of the experimental units prior to treatment with test substance. Second, confidence limits must be established for the measured parameters. Unique microcosms for each substance, or each dose rate are inapplicable. Third, reproducibility of parameters between control microcosms between comparable experiments must be reasonable.

Comparison of Reproducibility

Several comparable experiments have been conducted using the intact soil profile with and without aboveground vegetation. These experiments

Table 4.43. Indices of microbial activity measured at termination of a microcosm experiment using complex heavy metal emissions from a lead smelter (Ausmus et al., 1978)

Microbial parameter	Treatment	Soil	
		0.5 cm	5-10 cm
ATP concentration (ppm)	Contaminated	13.7 ^a	4.2 ^b
	Control	5.6	2.1
Bacterial density (10 ⁸ /g)	Contaminated	21.8 ^a	10.2 ^a
	Control	8.0	5.1
Fungal lengths (m/g soil)	Contaminated	75 ^a	135 ^a
	Control	1300	750

^aSignificantly different from controls at $P \leq 0.01$.

^bSignificantly different from controls at $P \leq 0.05$.

allow us to estimate reproducibility between experiments. An example of the findings is included as Table 4.44. We believe these microcosm units yield reproducible results as evidenced by the overlapping error terms for each of the three parameters among experiments.

Comparison of Replicability

Replicability among experimental units within a treatment class has been calculated during experiments using intact, homogenized, and sand-soil substrates. We have found that replicability is dependent upon substrate type and upon the parameter estimated. For example, CO₂ efflux was replicable in all substrates, but DOC efflux varied greatly among homogenized and sand-soil replicates at each sampling date and through time (Fig. 4.24). Similar results were obtained for nine nutrients (Table 4.43). Results from grass microcosms established using these substrates were similar. We conclude that replicability of intact soil microcosms is greater than other substrates.

4.5.1.4 Accuracy

Criteria

The criteria for determining microcosm result accuracy are dual. First, parameters indicative of ecosystem processes should be similar to those measured within comparable strata of the intact ecosystem. Second, the distribution and the transport rates of the test substance should mimic those which would occur in the source ecosystem. These criteria are the most difficult of those needed to establish the utility of microcosms in screening potentially hazardous substance. Only limited data have been collected to address this issue. The comparisons below represent our findings to date.

Table 4.44. Comparison of three parameters measured in control replicates of three soil core microcosm experiments

Experiment	Parameters		
	CO ₂ efflux ($\mu\text{g/day}$)	Concentrations in soil water	
		NO ₃ -N ($\mu\text{g/liter}$)	Ca ($\mu\text{g/ml}$)
1 ^a	5.73 $\pm$ 0.19	32.4 $\pm$ 4.8	20.3 $\pm$ 1.7
2 ^b	4.21 $\pm$ 0.35	30.1 $\pm$ 8.2	15.5 $\pm$ 5.6
3 ^c	3.17 $\pm$ 0.99	27.3 $\pm$ 9.2	18/6 $\pm$ 6.1

^aJackson et al., 1978.

^bAusmus and O'Neill, 1978; O'Neill et al., 1977.

^cAusmus et al., unpublished data.

ORNL-DWG 76-13428

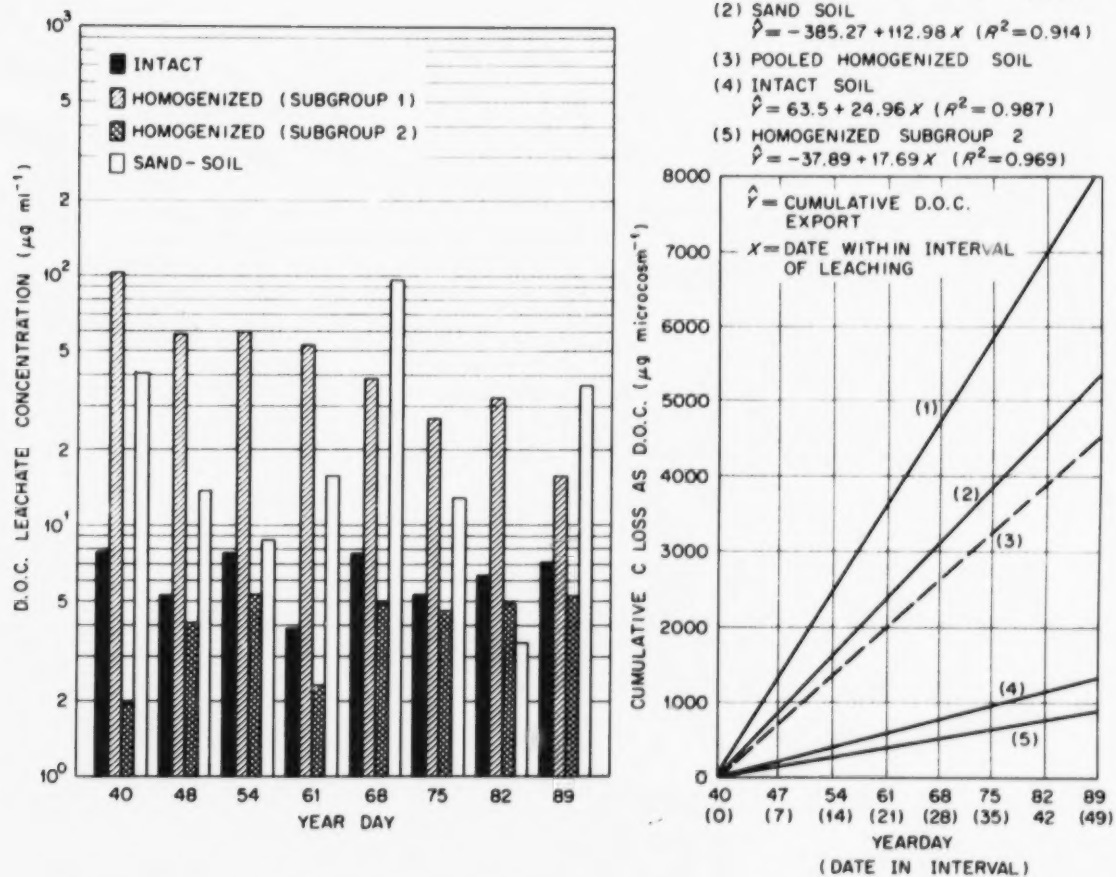


Figure 4.24. Replicability of two parameters monitored in microcosms. (A) - CO_2 efflux; (B) - DOC efflux (Ausmus and O'Neill, submitted).

Comparison of Accuracy

Export of essential elements in water leached from intact, homogenized and sand-soil substrate microcosms were extrapolated to one year and compared. Table 4.45 shows the relative loss from homogenized and sand-soil substrates compared to intact soil. If we assume that the standard is intact soil, the comparability of other substrate types is poor. No ion is similarly lost from the three substrate types.

Intact soil microcosms can be quantitatively related to ecosystems. Using data from the source ecosystem, transport of nutrients below the rooting zone of the forest (60-cm depth) was related to transport through intact soil microcosms (5-cm depth) by the expressions.

$$Y_1 = 0.184 X_1 ,$$

where Y_1 = DOC, $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$, or K transport to 60 cm depth ($\text{g/m}^2\text{/year}$), and

X_1 = DOC, $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$, or K transport to 5 cm depth in intact soil microcosms extrapolated to $\text{g/m}^2\text{/year}$.

$$Y_2 = 0.680 X_2 ,$$

where Y_2 = Ca, Na transport to 60 cm depth ($\text{g/m}^2\text{/year}$), and

X_2 = Ca, Na transport to 5 cm depth in intact soil microcosms extrapolated to $\text{g/m}^2\text{/year}$.

While these relationships alone are not conclusive proof of the accuracy of intact soil microcosm results, they are strong evidence that such is the case (Ausmus and O'Neill 1978, O'Neill et al. 1977).

Table 4.45 Comparison of extrapolated annual export among substrates
(O'Neill et al., 1977)

Substrate	Percent extrapolated intact soil microcosm transport							
	Ca	K	DOC	PO ₄ -P	NH ₃ -N	NO ₃ -N	Mg	Na
Homogenized								
Block 1	108	74*	76*	100	35**	180*	100	128
Block 2	168*	76*	366**	400**	43**	30**	138*	450**
Sand-soil	130*	20**	266**	20**	35**	18**	138*	200*

^aTransport extrapolated from intact soil (g/m²/year); Ca 5.8, K 5.4, DOC 6.2, PO₄-P 0.03, NH₃-N 0.66, NO₃-N 1.4, Mg 0.9, and Na 0.6.

* Significantly different from intact soil $P \leq 0.05$.

**Significantly different from intact soil $P \leq 0.01$.

More conclusive evidence was obtained from forest microcosm experiments (Jackson et al. 1978). Using the data shown in Table 4.3 and comparable data for cadmium, zinc, and copper, microcosm data were compared to the forest ecosystem under stress from the heavy metal deposition (see Watson et al. 1976, Jackson and Watson 1977). Enrichment ratios, ER, were computed by dividing the heavy metal concentration of the treated microcosm components by the same metal concentration of the control microcosm component. Greatest enrichment of metals occurred in litter strata. Cadmium had greater ER for soil than did other metals, although Pb was greatly enriched. Values compared to those near the heavy metal source were similar to the sampled ecosystem components (Jackson et al. 1978).

The most conclusive proof of accuracy to date has been the non-significant difference in field and microcosm data taken on similar dates.

4.5.1.5 Problems

Several problems remain in the evaluation of the utility of microcosms as screening tools to assess transport, fate, and effects of potentially hazardous substances. Four problems seem important. These are: (1) the rate of ecosystem degradation cannot be calculated from loss rates of nutrient elements as yet, although in theory it is possible to do so (O'Neill et al., in press); (2) the microcosms have not been satisfactorily tested using organic effluents, singly or in combination; (3) absolute accuracy of microcosm results has not been proven; and (4) reproducibility among microcosm studies conducted using intact profiles excised during different seasons has not been established.

Some information is needed to conduct adequate screening of potentially hazardous substances. The mode of entry of the substance into the system is needed. The direct toxicity of the substance to research workers is needed in order to practice safe laboratory experiments. The potentially targeted ecosystems should be identified if possible and used as sources of microcosm units. Lacking this information, forest or pasture systems should be used because more than 80% of the non-urban United States is one of these ecosystems.

High mobile invertebrates, macroinvertebrates, and vertebrates are not well represented within these small microcosm units. Studies directed toward the potential effects of these substances on these, due to their economic importance or trophic position, should be conducted as biomagnification experiments.

4.5.1.6 Recommendations

Based on the several experiments conducted to date, we recommend the use of intact soil microcosms excised from targeted ecosystems and encased with or without aboveground vegetation, depending upon experimental purpose. We cannot recommend the use of artificial substrates, or homogenized soil reinoculated with biota for several reasons, including: (1) instability of these substrates with respect to parameters monitored; (2) limited replicability and accuracy of these units; and (3) proportionally higher cost in experimental set-up.

There is no substitute for choice of the appropriate experimental design for the experiment to be performed. We have found that a randomized incomplete block design with incomplete factorial treatment arrangement is cost-effective and information rich. Analysis of

parameter behavior through time under control and stressed conditions may furnish additional insights into the mode of effects on ecosystem processes and on response and recovery times which can be expected to levels of the stressor (Van Voris et al. 1978).

4.5.2 Protocol for Construction and Operation of Terrestrial Microcosms (B. S. Ausmus and D. R. Jackson)

4.5.2.1 Objectives

The purpose of these protocols is to provide an accurate, rapid, cost-effective method to assess contaminant transport, fate, and effects upon terrestrial ecosystems likely to be impacted. The assessment is two-tiered. The first protocol provides a rapid, binary (yes/no) solution to assessment questions and emphasizes the relationship between dose and contaminant transport and effects. The second protocol is more extensive, and allows quantitative assessment of rates of transport, accumulation, and nutrient cycling disruption.

4.5.2.2 Rationale

The initial question when faced with a potentially hazardous substance is whether it is mobile, toxic, or disruptive of ecological processes at various dosages. After determining whether these do occur, and if so, at what doses, then one must ask a battery of questions (within a second protocol), including at what rates transport and accumulation occur.

Previous studies have shown that excised, intact soil blocks with (protocol 2) or without (protocol 1) plants are the most accurate,

replicable, and cost-effective microcosm units for contaminant assessment (O'Neill et al, submitted; Ausmus and O'Neill, submitted). Protocol 1 uses intact soil cores without plants to assess dose/mobility and dose/effects relationships, exploiting the fact that terrestrial ecosystem processes are predominantly belowground. Protocol 1 should answer specifically:

- (1) Is the contaminant (or some by-product) mobile?
- (2) Is the primary mode of soil export gaseous, dissolved, or particulate?
- (3) Is nutrient cycling disruption indicated?
- (4) Are soil biota affected?
- (5) Do questions 1 to 4 change as a function of dose?

Previous studies have indicated that assessment of whole system transport, fate, and effects is more time-consuming and expensive than assessment of soil processes. Protocol 2 addresses quantitative assessment of transport, fate, and effect of contaminant under study throughout the ecosystem. Specifically, we will use protocol 2 to answer:

- (1) What is the rate of contaminant transport?
- (2) What transformation/degradation products result and how does their mobility, etc., compare with that of the parent compound?
- (3) How fast does nutrient/carbon depletion occur? Is this effect sustained or does recovery occur?
- (4) Where are contaminant accumulation sites?
- (5) What is the rate of plant uptake?

(6) How does the contaminant partition among biotic and abiotic components?

(7) What is the effect upon: (a) plant biomass, (b) microbial community dynamics, and (c) invertebrate community dynamics?

In order to obtain the most useful information from either protocol, it is preferable to use cores extracted from those terrestrial systems most likely to be the target of contaminants under assessment. These protocols were developed for forest and grassland, since ~ 80% of U.S. landscape is one or the other of these. We believe, lacking identification of target ecosystems, that use of cores from convenient unimpacted forests or grasslands will yield results representative of these ecosystem types, regardless of the specific microclimatic regime.

We cannot recommend use of artificial substrates or homogenized soil that have been reinoculated with biota, for several reasons. Most important of these are: (1) these substrates are unstable with respect to the parameters being monitored with/without perturbation; (2) they are less replicable and reproducible than intact systems; and (3) their accuracy for assessing environmental transport rates, accumulation rates, and effects (especially on ecosystem processes) is unpredictable (Ausmus and O'Neill, submitted; Jackson et al., submitted)

Careful core excision, encasement, and equilibration will allow users high quality control over contaminant assessments. Units have been maintained for protocols 1 and 2 under microcosm conditions for long intervals (three months to two years). Therefore, many units could be prepared and equilibrated and used as needed.

4.5.2.3 Protocol 1

(1) Obtain soil cores from representative terrestrial ecosystems, 5-cm diam x 5-cm depth; remove aboveground plant parts.

(2) Encase the cores in shrinkable polyvinyl chloride (or teflon, 2/1000 in., within shrinkable PVC if contaminant is organic) and gently heat shrink until a tight bond with the core (minimum boundary flow) is achieved. Leave enough lining above the soil surface to use gaseous export traps, if necessary. Mount on glass funnels in test tubes. Blacken core wrapping to negate abnormal algae growth. Place in environmental chamber under as near the field conditions as possible (see Fig. 4.25).

(3) Equilibrate two to three weeks. Leach with rainwater (known water chemistry) three to five times (enough to obtain 20-30 ml/date during equilibration. If possible, determine dissolved organic carbon (DOC) concentrations in these samples. If possible use alkali traps to determine daily CO_2 efflux eight to ten days during equilibration. Use these data to discard dissimilar replicate soil cores.

(4) Experimental procedure: randomized complete block and, if possible, factorial treatment arrangement of dosages with a minimum of three cores per dose per terrestrial ecosystem tested. Dosages of a wide range should be used for this rapid screening test to maximize the clarity of dose dependent observations.

(a) Add contaminant, if possible, as a uniformly labeled radiolabelled compound so that transport of any derivative can be determined with minimal chemical analysis. If possible, add contaminant in the mode in which it would be expected to enter the ecosystem, e.g., particulate deposition.



Figure 4.25. Protocol 1 soil core excised intact from a forest ecosystem.

(b) Set gas traps to monitor CO_2 efflux and gaseous emission of contaminant. Standard alkali traps, such as 0.2 N KOH can be used for CO_2 recovery. Contaminant volatility, solubility, and chemical detection methods will determine methods specifically used for each contaminant tested. Collect data at 24-hr intervals.

(c) On Day 6, add sufficient rainwater (known water chemistry) to collect 20-30 ml leachate per core. Analyze DOC concentration. Determine contaminant concentration (if possible by radioassay techniques).

(d) On Day 9, repeat step (c). After leachate collection, harvest microcosm units. Cut cores into 1-cm depth intervals. Within each depth, determine contaminant concentration, biotic activity by ATP assay, and/or adenylate energy charge (Ausmus, in press; Ching and Ching 1972).

(5) Interpretation of results from protocol 1 will allow user to discern: (1) dose relationship to transport and effect measurements; and (2) major modes of transport (gaseous, dissolved), if transport is significant.

4.5.2.4 Protocol 2

(1) Obtain intact soil blocks containing plants (grassland:circular: 15-cm diam x 10-cm depth; forest:square 40- x 20-cm depth, containing a shallow-rooted plant such as red maple).

(2) Encase as in protocol 1 with this modification. Grassland: use a base of Plexiglas (inorganic contaminant or glass, if organic

contaminant) ported in the center with plastic or glass. Place encased microcosm in container and fill around microcosm with ignited acid plus distilled water-washed sand. Place on platform and attach leachate bottle (see Fig. 4.26); maintain in an environmental chamber. Forest microcosms should be encased in polyethylene or teflon (2/1000 in.) and sealed tightly outside by placing in a plywood box and filling with concrete (Fig. 4.27). Leachate ports can be placed at the side. If a suitable site is available, maintain these microcosms out-of-doors.

(3) Equilibrate three to five weeks. Leach weekly as in protocol 1. If possible, monitor CO_2 efflux daily. Determine concentrations of four nutrients in leachate: (a) grassland:DOC, NH_4 , PO_4 , Ca; (b) forest:DOC, NO_3 , PO_4 , Ca. Use these data to determine individual microcosm response to treatment after contaminant addition.

(4) Experimental procedure: randomized complete block with a minimum of three replicates and three doses per ecosystem.

- (a) Add contaminant stable and (if feasible) radiotracer.
- (b) Set gas monitoring traps as protocol 1.
- (c) Leach weekly. Determine nutrient (four elements above) and contaminant concentrations.
- (d) Take plant tissue samples for determination of contaminant concentration weekly.

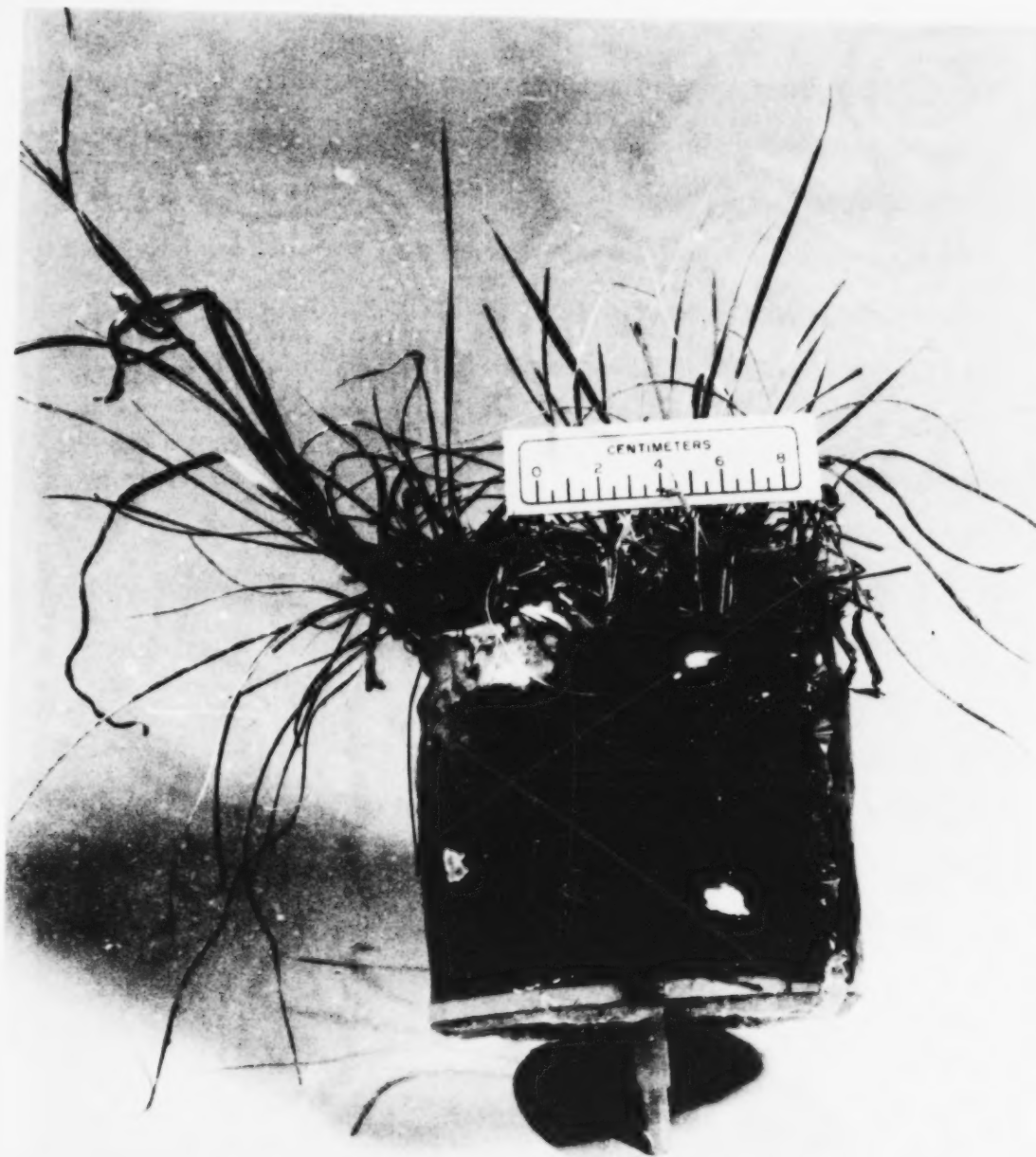


Figure 4.26. Protocol 2 grassland microcosm excised from a *Festuca*-dominated pasture. Encased microcosm showing heat-shrunk PVC, Plexiglass disk and leachate port. Photograph taken nine months after encasement.



Figure 4.27. Protocol 2 forest microcosm containing an Acer rubrum sapling excised from a mesic hardwood forest.

(e) At eight to ten weeks, harvest microcosm units; analyze:
(i) extractable nutrients using 1 M KCl, 1 M NaHCO₃ intact extraction procedure (Jackson and Hall 1978); (ii) above and belowground plant contaminant concentration, biomass; (iii) biotic activity (ATP and/or adenylate energy charge); (iv) bacterial density (dilution plating); (v) fungal biomass (Jones-Mollison slides); (vi) nematode and microarthropod density and species composition (density gradient flotation) (Parkinson et al. 1971; Phillipson 1971); (vii) contaminant and daughter compounds concentrations in 1 cm depth of soil.

(5) Interpretation of results from protocol 2 will allow us to
(a) quantify transport rates, (b) determine longevity of nutrient depletion, (c) calculate plant contaminant uptake rates; and (d) quantify soil and plant populations affected by contaminant.

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APPENDIX A
SYNOPSIS OF AQUATIC EXPERIMENTS

EXPERIMENT 1. MICROCOSM SIZE AND SUBSTRATE TYPE; ARSENIC TRANSPORT

Objectives

- (1) To establish basic microcosm techniques (set-up, sampling, and monitoring procedures).
- (2) To identify parameters of interest for the measurement of ecosystem properties, such as dissolved oxygen, water chemistry, and pH.
- (3) To determine the effect of size [7-liter (1.85 gal) vs 70-liter (18.5 gal)] on ecosystem properties, microcosm stability and persistence, and replicability.
- (4) To determine the effect of sediment type (sand vs lake sediment) on ecosystem properties, microcosm stability and persistence, and replicability.
- (5) To measure the accumulation of ^{74}As (in tracer amounts) in various ecosystem components, and to assess effects of size and sediment type on arsenic transport.

Timetable

January 12-February 19, 1976	Microcosms assembled.
February 19	Began biological and chemical sampling, and routine monitoring of pH, temperature, conductivity, and dissolved oxygen.
February 23	One 70-liter sand microcosm burst and was replaced.

April 12

Sodium arsenate added to all microcosms. Began monitoring ^{74}As in water.

May 18-19

Final sampling; termination of experiment.

Microcosm Construction

Six 7-liter and six 70-liter microcosms were assembled. Approximately 20 kg of sediment was collected from Watts Bar and Melton Hill reservoirs (Tennessee), dried at 105°C in a forced-air drying oven, and ground with a mortar and pestle. The same amount of Ottawa sand was rinsed in distilled water and oven-dried. On January 12, 1976, approximately 3.5 cm of sand or sediment was placed in each aquarium, with three replicates of each size-substrate combination. Spring water was then added slowly. A minor catastrophe occurred after three of the large aquaria had been filled--the supporting rack in the environmental chamber collapsed under the weight, and the completion of the microcosms was delayed for four weeks while stronger tables were constructed. On February 11, filling was resumed. The lake sediment tanks were murky at first due to suspended clay particles, but gradually cleared over the next seven days.

On February 19, filamentous algal communities were collected from a concrete fish holding tank outside the Environmental Sciences Laboratory. The algae were drained in a sieve; six 30-mg and six 6-mg portions were weighed out and placed in the 70-liter and 7-liter microcosms, respectively. Ten large Physsa (total live wt approximately 10 g) were placed in each large tank, and two in each small tank.

On February 23, one of the large sand microcosms burst, and was reconstructed following the same procedure.

Parameters Measured

pH
temperature
conductivity
dissolved oxygen
concentrations of major ions (Na, K, Ca, Mg, HCO_3 , Cl, SO_4)
nutrient concentrations (NO_3 -N, NH_4 -N, PO_4 -P, Total P, Fe)
dissolved organic carbon
final algal biomass
final chlorophyll (in water)
final sediment ATP
dominant organisms (qualitative)
arsenic-74 in water, substrate, algae, and snails

Further Information

For a description of sampling and radioassay techniques, see Section 3.2.2.1. This section also presents results on arsenic transport. Other results of Experiment 1 are discussed in Sections 3.2.1.3., 3.2.1.4., and 3.2.1.5. The experiment was the subject of a paper presented at the 1976 AIBS Annual Meeting in New Orleans, and later published in the open literature (Giddings and Eddlemon 1977).

EXPERIMENT 2: DOSAGE EFFECTS--ARSENIC

Objectives

(1) To determine the effect of dosage level on the transport and distribution of arsenic.

(2) To determine the effects of three arsenic dosages on biological and chemical parameters in microcosms.

(3) To repeat a portion of Experiment 1 for assessment of the reproducibility of microcosm results.

Timetable

June 29-July 6, 1976	Twelve microcosms assembled.
July 6	Began biological and chemical sampling, and routine monitoring of pH, temperature, conductivity, and dissolved oxygen.
July 8	One tank burst and was replaced.
August 18-19	24-hr production, respiration experiments.
August 25-26	
September 16-17	
September 17	Sodium arsenate added to 9 microcosms (3 concentrations x 3 replicates; plus 3 controls).
September 20	Began monitoring ^{74}As in water and sediment.
September 16-17	Began weekly production and respiration experiments.

November 7-12

Final sampling.

November 24

Experiment terminated.

Microcosm Construction

Sediment was shoveled from the bottom of a shallow pond (mean depth approximately 1 m) behind the ORNL Aquatic Ecology Laboratory, on June 29, 1976, and carried into the environmental chamber in tubs. On July 1, an 8-cm layer of sediment was placed into each of 12 70-liter aquaria. Spring water was added to three of the tanks on July 1, and to the other nine on July 6. A mixed Elodea-Potamogeton community was collected from the pond, drained, and 40-g aliquots placed in each microcosm on July 6. As in Experiment 1, one of the aquaria burst a few days after the experiment began, and was replaced.

Parameters Measured

pH

temperature

conductivity

dissolved oxygen

concentrations of major ions (see Experiment 1)

concentrations of nutrients (see Experiment 1)

dissolved organic carbon

net primary production

total ecosystem respiration

dominant organisms (qualitative)

arsenic-74 in water, sediment, and biota

EXPERIMENT 3: POND ENCLOSURES

Objectives

(1) To compare the ecological characteristics of pond communities enclosed in situ with conditions in the surrounding pond and in microcosms.

(2) To obtain field data on arsenic transport for comparison with Experiment 3.

Timetable

July 15, 1976	Six Plexiglas enclosures placed in pond.
July 19	Began chemical sampling, and routine monitoring of pH, temperature, conductivity, and dissolved oxygen.
September 17	Sodium arsenate added to 3 enclosures.
September 21	Began monitoring ^{74}As in water.
October 26-28	Final sampling.

Description of Enclosures

Six enclosures were constructed out of Plexiglas. The enclosures measured 60 x 120 cm and were 91.4 cm high. The corners were reinforced with square Plexiglas rods, and two Plexiglas strips were fastened between the long sides at the upper edge of each enclosure for added stability. Each enclosure had two handles at each end.

The enclosures were positioned in a shallow pond (the source of the microcosm components for Experiment 2) by two men wearing waders. The pond had an irregular rocky bottom, overlain by 5 to 10 cm of sediment. Because of the uneven bottom, it was difficult to push the enclosures evenly into the sediment, and many possible locations were tried before six suitable sites were found. The enclosures extended about 2 to 5 cm below the sediment surface and above the waterline.

Parameters Measured

pH

temperature

conductivity

dissolved oxygen

concentrations of major ions (see Experiment 1)

nutrient concentrations (see Experiment 1)

dissolved organic carbon

arsenic-74 in water, sediment, and biota

EXPERIMENT 4: FIRST PROTOCOL TEST--CHROMIUM

Objectives

(1) To test our aquatic microcosm protocol with a second inorganic contaminant.

(2) To evaluate the production/respiration ratio as an indicator of damage to aquatic ecosystems.

(3) To continue development of techniques for studying trace element transport in microcosms.

Timetable

January 12, 1977	Eight microcosms assembled.
January 19-20	Began monitoring production and respiration with Dissolved Oxygen Monitoring System.
January 25	Began routine monitoring of pH, temperature, and conductivity.
February 9	Snails added to microcosms.
April 12	Sodium chromate added to 6 microcosms. (3 concentrations x 2 replicates; plus 2 controls).
April 27	
May 11	
May 25	
April 12	Began monitoring chromium in water and sediment.
June 9-10	Final sampling; experiment terminated.

Microcosm Construction

Sediment was collected from the Clinch River, just below Melton Hill Dam, on January 12, 1977. Within hours, a 5-cm layer was placed in each of eight 7-liter aquaria. The aquaria were then filled with spring water. A 6.0-g (drained wet wt) aliquot of a filamentous algal community from Target Range Pond was added to each microcosm. On February 9, five adult snails (Physa) from a large fish culture tank were placed into each microcosm (total live wt 0.77-0.85 g).

Parameters Measured

pH
temperature
conductivity
dissolved oxygen
net primary production
total ecosystem respiration
chromium in water, sediment, and biota

EXPERIMENT 5: FISH MICROCOSMS

Objective

To investigate the consequences of adding fish to the standard microcosm design.

Timetable

June 30, 1977	Eight microcosms assembled.
July 7	Fish added.
July 15	Began monitoring pH, temperature, conductivity, and dissolved oxygen; chemical and biological sampling.
October 3	Bluegill and <u>Gambusia</u> microcosms treated with ^{74}Se (Experiment 6).
October 14	Began monitoring net primary production and total ecosystem respiration with dissolved Oxygen Monitoring System.
November 22	Bluegill and <u>Gambusia</u> microcosms terminated (Experiment 6).
December 12	Control microcosms treated with ^{203}Hg (Experiment 7).
March 17, 1978	Remaining microcosms terminated.

Microcosm Construction

All microcosm components (except fish) were collected on June 30, 1977, from Target Range Pond, a shallow (1-m) spring-fed pond on the Oak Ridge Reservation. Water was collected in large polyethylene containers near the outlet of the pond. Sediment and an Elodea community were collected from the center of the pond. The sediment was distributed among eight 70-liter aquaria; the sediment depth was 5 cm. Pond water was then slowly siphoned into each aquarium, and 100 g (drained wet wt) of the Elodea community was added.

Fish were introduced on July 7. Gambusia were collected from a stocked pond, and bluegill and fathead minnows were selected from laboratory cultures. The fish additions were as follows:

<u>Microcosm number</u>	<u>Fish added</u>	<u>Total wet wt</u>
M1	10 <u>Gambusia</u>	0.36 g
M2	"	0.63 g
F1	5 fathead minnows	0.68 g
F2	"	0.68 g
B1	2 bluegill	7.8 g
B2	"	8.1 g
C1	None (controls)	----
C2	"	----

Parameters Measured

pH

temperature

conductivity

dissolved oxygen

concentrations of major ions (see Experiment 1)

nutrient concentrations (see Experiment 1)

dissolved organic carbon

net primary production

total ecosystem respiration

dominant organisms (qualitative)

EXPERIMENT 6: SELENIUM TRANSPORT

Objectives

- (1) To examine the movement of selenium in pond microcosms containing fish.
- (2) To increase the amount of information on contaminant transport which can be obtained from a microcosm experiment.

Timetable

June 30, 1977	Microcosms assembled as part of Experiment 5 (Microcosms B1, B2, M1, and M2).
October 3	Bluegill and <u>Gambusia</u> microcosms from Experiment 5 treated with sodium selenate. Began monitoring ⁷⁵ Se in water, sediment, <u>Elodea</u> , and snails.
November 18-22	Final sampling.
November 22	Experiment terminated.

Microcosms

See Experiment 5.

Parameters Measured

selenium-75 in water, sediment, and biota
(see also Experiment 5)

EXPERIMENT 7: MERCURY TRANSPORT

Objectives

- (1) To examine the movement of mercury in pond microcosms.
- (2) To compare the behavior of mercury with that of the other trace elements tested (arsenic, chromium, and selenium).

Timetable

June 30, 1977	Microcosms assembled as part of Experiment 5 (Microcosms C1 and C2).
December 12	Control microcosms from Experiment 5 treated with mercuric nitrate. Began monitoring ^{203}Hg in water, sediment, <u>Elodea</u> , and snails.
January 18-25, 1978	Final sampling.
March 17	Experiment terminated.

Microcosm Construction

See Experiment 5.

Parameters Measured

mercury-203 in water, sediment, and biota
elimination of mercury by snails
(see also Experiment 5)

EXPERIMENT 8: ANTHRACENE TRANSPORT AND FATE

Objectives

- (1) To test the standard pond microcosm design with an organic contaminant.
- (2) To study the transport and chemical fate of anthracene in pond microcosms.
- (3) To compare microcosm results with data collected by conventional contaminant testing methods.

Timetable

January 7-12, 1977	Two microcosms assembled
January 19-20	Began monitoring production and respiration with Dissolved Oxygen Monitoring System.
January 25	Began routine monitoring of pH, temperature, and conductivity.
July 13	Anthracene added. Began monitoring anthracene and derivatives in water, sediment, and biota.
October 5-6	Final sampling.
October 6	Experiment terminated.

Microcosm Construction

Sediment and a filamentous algal community were collected from Target Range Pond on January 7, 1977. On January 10, approximately 6 cm of sediment were placed in each of two 70-liter aquaria. The

aquaria were then filled with spring water. Two days later, unweighed portions of the algal community (approximately 10 g) were added to each microcosm.

Parameters Measured

pH

temperature

conductivity

dissolved oxygen

net primary production

total ecosystem respiration

dominant organisms (qualitative)

anthracene and derivatives in water, sediment, and biota

EXPERIMENT 9: SUCCESSION AND NUTRIENT CYCLING

Objective

To observe changes in community structure, ecosystem metabolism, and nutrient cycling during the first two months following construction of a pond microcosm.

Timetable

November 14, 1977	Two microcosms assembled. Began biological and chemical sampling and routine monitoring of pH, temperature, and conductivity.
November 15	Began monitoring net primary production and total ecosystem respiration with the Dissolved Oxygen Monitoring System.
March 17, 1978	Experiment terminated.

Microcosm Construction

Water, sediment, and an Elodea community were collected from Target Range Pond on November 14, 1977, as in Experiment 5. About 4 cm of sediment was placed in each of two 70-liter aquaria. One Amicon^R hollow fiber interstitial sampling unit was placed on the sediment in each microcosm, and covered with an additional 2 cm of sediment. Pond water was then slowly siphoned in. A portion of the Elodea community (100 g drained wet wt) was added to each microcosm.

Parameter Measured

pH

temperature

conductivity

dissolved oxygen

nutrient concentrations (see Experiment 1) in water and
interstitial water

net primary production

total ecosystem respiration

dominant organisms (qualitative)

Microcosm Biota

Algae

Blue-greens

- I. Chroococcales
 - 1. Aphanocapsa
 - 2. Coelosphaerium
 - 3. Gomposphaeria
 - 4. Merismopedia
 - 5. Chroococcus
 - 6. Microcystis
- II. Oscillatoriales
 - 7. Oscillatoria (Lyngbya)
- III. Nostocales
 - 8. Anabaena
 - 9. Cylandrospermum

Green algae

- IV. Volvocales
 - 10. Carteria
 - 11. Chlamydomonas
 - 12. Gonium
 - 13. Pandorina
- V. Tetrasporales
 - 14. Gloeocystis
- VI. Chlorococcales
 - 15. Planktosphaeria
 - 16. Tetraedron
 - 17. Palmella
 - 18. Sphaerocystis
 - 19. Ankistrodesmus
 - 20. Chlorella
 - 21. Nephrocystium
 - 22. Oocystis
 - 23. Selenastrum
 - 24. Golenkinia
 - 25. Actinastrum
 - 26. Coelastrum
 - 27. Scenedesmus
 - 28. Pediastrum
- VII. Ulotrichales
 - 29. Geminella
 - 30. Ulothrix
- VIII. Chaetophorales
 - 31. Aphanochaete
 - 32. Coleochaete
- IX. Oedogoniales
 - 33. Oedogonium

- X. Cladophorales
 - 34. Cladophora
 - 35. Pithophora
 - 36. Rhizoclonium
- XI. Zygnematales
 - 37. Mougeotia
 - 38. Spirogyra
 - 39. Zygnema
 - 40. Closterium
 - 41. Cosmarium
 - 42. Pleurotaenium
 - 43. Staurastrum

Euglenophytes

- XII. Euglenales
 - 44. Euglena
 - 45. Phacus
 - 46. Menoidium

Dinoflagellates

- XIII. Dinokontae
 - 47. Gymnodinium

Cryptophytes

- XIV. Cryptomonadaceae (family)
 - 48. Cryptomonas

Chrysophytes

- XV. Ochromonadales
 - 49. Dinobryon
 - 50. Mallomonas
 - 51. Synura

Diatoms

- XVI. Centrales
 - 52. Melosira
- XVII. Pennales
 - 53. Asterionella
 - 54. Fragilaria
 - 55. Meridion
 - 56. Synedra
 - 57. Tabellaria
 - 58. Eunotia
 - 59. Cocconeis
 - 60. Gomphonema
 - 61. Amphora
 - 62. Epithemia
 - 63. Rhopalodia
 - 64. Unknown A
 - 65. Unknown B

PROTOZOA

- XVIII. Sarcodina
 - 66. Amoeba
 - 67. Diffugia
 - 68. Heliozoa
- XIX. Mastigophora
 - 69. Oikomonas
 - 70. Other flagellates
- XX. Infusoria
 - 71. Coleps
 - 72. Lacrymaria
 - 73. Prorodon
 - 74. Loxophyllum
 - 75. Chilodon
 - 76. Urocentrum
 - 77. Colpidium
 - 78. Uronema
 - 79. Glaucoma
 - 80. Paramecium
 - 81. Lembadion
 - 82. Cyclidium
 - 83. Spirostomum
 - 84. Stentor
 - 85. Stichotricha
 - 86. Uroleptus
 - 87. Oxytricha
 - 88. Stylonychia
 - 89. Euplotes
 - 90. Vorticella
 - 91. Other ciliates

MACROFAUNA

- XXI. Hydra
- XXII. Turbellaria
- XXIII. Nematoda
- XXIV. Rotatoria
- XXV. Gastrotricha
- XXVI. Oligochaeta
- XXVII. Cladocera
- XXVIII. Copepoda
- XXIX. Ostracoda
- XXX. Hydrocarina
- XXXI. Gastropoda
- XXXII. Insecta
- XXXIII. Vertebrata

APPENDIX C
PROJECT PUBLICATIONS

PROJECT PUBLICATIONS

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